FEB 1958
LIBRARY
FISH & WILDLIFE SERVICE

JOURNAL

OF THE

TOKYO UNIVERSITY OF FISHERIES

VOL. 43, NO. 2.

CONTENTS

NONAKA, J.: Studies on the chemical essentials to the Discoloration of fish oil 127–165
OSAKABE, I., HIRANO, T. and OKADA, I.: Studies on the Kamaboko made by the enriching of Vitamins 167–172
FUKUSHIMA, K., OSAKABE, I., KIKUCHI, T. and OKADA, I.: Polarographic studies on fish muscle meats 173–178
OSAKABE, I., FUKUSHIMA, K., KIKUCHI T. and OKADA, I.: Polarographic studies on the cuttle fish meat. Part II 179–181
SEKINE, Y., TAKEUCHI, J. and INOUE, K.: Investigation on the source and area of contamination in Tokyo Bay 183–197
HONDA, K., KIKUCHI, T. and OKADA, I.: Atmospheric Exposure of netting threads fouled with fatty acid, linseed oil and sardine oil.. 199–205
YOSHIHARA T.: Population studies on the Japanese ivory shell, *Babylonia japonica* (Reeve) 207–248
KUBO, I. and ASADA, E.: A quantitative study on crustacean bottom epifauna of Tokyo Bay 249–289

THE TOKYO UNIVERSITY OF FISHERIES
SHIBA KAIGANDŌRI 6, MINATO-KU, TOKYO, JAPAN
March, 1957.

JOURNAL OF THE TOKYO UNIVERSITY OF FISHERIES

Editors for this Journal

Ikunosuke Okada, Professor, Dr.

Hiroshi Niino, Professor, Dr.

Itsuo Kubo, Professor, Dr.

Journal of the Tokyo University of Fisheries, Vol. 43, No. 2, 1957

STUDIES ON THE CHEMICAL ESSENTIALS TO THE DISCOLORATION OF FISH OIL

Junsaku NONAKA

Contents

Preface 127
Chapter I. Discoloration Mechanism of Fish Oil 128
Section 1. Final Necessary Condition for the Discoloration 128
Section 2. The Second Factor 132
Section 3. The Constituents of Oil Having Relation to the Discoloration 137
Remarks on the Function of Second Factor 139
Chapter II. Studies of the Oxidized Acids 140
Section 1. Oxidized Acids as an Essential Coloring Component of Discolored Oil 140
Section 2. Chemical Characteristics of the Oxidized Acids 144
Section 3. Fractionation of Oxidized Acids 146
Section 4. Correlation of the Oxidized Acids to the Drying-Phenomenon of Oil 149
Remarks about the Effect of Sunlight on the Discoloration 152
Chapter III. Identification of the Colored Entity 153
Section 1. Mother Substance of Colored Material in Discolored Oil 153
Section 2. Behaviors of Alkali and Acid towards Carbonyl Compounds .. 154
Section 3. Paper Chromatography of Acidic Carbonyl Compounds 158
Section 4. Detection of Half Aldehyde in Autoxidized Oils 160
Summary 162
References 164

PREFACE

Most edible fats and oils obtained from terraneous plants or land animals, though they become shadowed by the occurrence of rancidity when exposed to air, scarcely suffer "rusting" i.e., a deterioration accompanied by a strong discoloration. On the other hand, in the case of such from aquatic livings, especially of fish oil, manufacturers are troubled not only with the problem of rancidity but very often with the fact that their commodities become badly discolored through rusting. So, it is one of the most serious problems in manufacturing marine products how to evade this discoloration.

However, despite the importance of the problem, relatively little has been declared about the fundamental items of this phenomenon, e.g., reaction mechanism, nature of the colored substance, etc., the apprehension of which is expected to render good service for attaining a proper means of preventing this unwellcomed affair.

In this paper the author describes several factors underlying the phenomenon, into which he has made some systematic inspections from the experimental point of view.

The author whishes to express his sincere gratitude to Dr. T. MORI, Professor of the Tokyo University, for his kind supervision and encouragement bestowed throughout the course of the present study. The author is also indebted to the late Dr. T OYA, Dr. M. MIGITA, and Dr. Y. OBATA for their very invaluable advices. The author wishes to express his hearty thanks to Messrs. T. MIZUNO, S. NISHIGAKI, Y. GOTO, M. KOMATSU, and K. ANDO for their participation in this study. He is also under an obligation to the Laboratory of Nippon Oil Co. Ltd., the Laboratory of Takasago Perfumery Co. Ltd., and Dr. H. HIRANO of the Tokyo University of Fisheries, for their kindness shown in various ways.

The present study was supported in part by a Grant in Aid for Departmental Scientific Research from Ministry of Education and from Ministry of Forestry to which he offers his hearty acknowledgment.

CHAPTER I. DISCOLORATION MECHANISM OF FISH OIL

Section 1. Final Necessary Conditions for the Discoloration

So far as foreign countries are concerned it will be too much to say that only a few studies have been announced about the rusting of fish oil, while almost all of them treat of the discoloration phenomena exclusively of white paint or soap.

Surveying these publications, more than ten of factors can be numerated that seem to have a positive effect upon the process of the discoloration, viz. volatile basic nitogen compounds such as ammonia, trimethylamine[1)-6)], etc., basic oxides of metals[7)8)], other basic compounds[6)], 2-naphthol[9)], ferrous oleate[10)], pyridine[11)], mono-, di-, or tri-chloroacetic acid[11)], mucilaginous matters[9)12)], blood[13)], hemoglobin[13)14)], trace of NO_2[15)], casein[16)], fish-meal[16)], autoxidation[14)15)16)17)18)], advanced degree of unsaturation of the oil[7)12)16)19)20)21)22)], darkness[7)8)16)18)23)24)25)26)], etc. These papers, however, do not always agree with each other as to the indispensability of these respective factors; and no attempt has ever been made to establish final and definite conditions that are essential to the occurrence of discoloration.

On the basis of experimental results given below the author builds up a conception that the first condition necessary for the discoloration to occur is that the oil submits to autoxidation to some extent, but mere oxidation hardly results in discoloration; and if a certain factor, such as volatile basic nitrogen compound, comes into contact with this oxidized oil, then begins the discoloration to proceed. The author defines such materials, therefore, as the second factor in the discoloration mechanism.

As is shown in Exp. 1, fresh samples of fish oil stored in air and in contact with casein, glycine, etc., become discolored first when their gain in weight has exceeded 4 to 5% of their initial weight. On the contrary, when fresh oil samples are kept alone or in contact with sodium chloride, dextrin, or gelatin, respectively, they exhibit no appreciable discoloration even after autoxidation has advanced profoundly. Therefore, it may be said that, whereas autoxidation is

not to be disregarded of its effect as a premise to the discoloration, something beside autoxidation is required for the phenomenon to become visible.

Another evidence for the fact that autoxidation is virtually nothing but the first necessary condition will be given in Exp. 2. Thus, as shown in Table 1, when fresh fish oil is mixed with casein, which is to act as a second factor, and kept exposed to air, it undergoes discoloration, but if beforehand pyrogallol as antioxidant is added by 1% to the oil, discoloration occurs hardly.

Furthermore, as shown in Exp. 3 (see Table 2), it is clear that the more strongly the oil has been oxidized, the deeper color is developed when met with a second factor like ammonia.

Experiment 1.

Every about 100mg of fresh sardine-oil were put spread on dried stripes of filter paper, 5cm×2cm, which had been kept beforehand in distilled water, or in 2% aqueous solutions of, respectively, casein, gelatin, glycine, cystine, dextrin, and sodium chloride. The color change of the oil was observed in dispersed light in parallel to the gain in weight of the oils at 4~8°C. The course of the gain in weight is shown for a few stripes in Figure 1. The discoloration of all the oils, kept in contact with casein, glycine, and cystine, respectively, became apparent with one accord when about 60~70 hours had elapsed after the initiation of the experiment, these periods corresponding to 4~5% increase in weight of the oils, while the oil which had been kept in contact with gelatin, dextrin, NaCl, or without any concomitant did not discolor at all even after 80 hours.

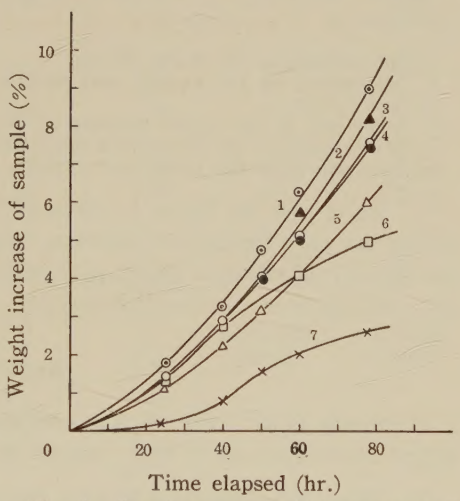


Fig. 1. Spontaneous weight increase of oil kept in contact with respectively: 1. NaCl, 2. gelatin, 3. glycine, 4. cystine, 5. casein, 6. starch, 7. control.

Experiment 2.

Samples from a definite stock of fresh shark liver oil, the one being intact (control) and the other protected from autoxidation by adding 1% pyrogallol*, were mixed equally with 5 parts of casein, which had previously been thoroughly washed with ether and kept stored for three months at room temperature. After that, chemical characteristics as well as color of the oil samples were examined. The results obtained are shown in Table 1.

* It has been verified that the action of pyrogallol in this case is only to prevent the intact oil from undergoing autoxidation. (Bul. Japan. Soc. Sci. Fish. **15**, 270 (1949); **18**, 109 (1952).

Table 1. The effect of autoxidation on the degree of discoloration of oil.

	Intact oil	After three months storage			
		Without pyrogallol		With 1% pyrogallol	
		In the dark	In dispersed light	In the dark	In dispersed light
Color	yellow	reddish brown	reddish orange	yellowish orange	yellow
Viscosity	—	very viscous	very viscous	slightly viscous	slightly viscous
Acid value	0.56	72.27	80.76	37.40	25.41
Sapon. value	164.0	285.4	286.1	193.4	194.1
Iodine value[1]	167.6	57.17	52.7	155.5	158.1
Peroxide oxygen[2]	21.8	375.8	395.7	54.5	79.3
KREIS-test	0	3.4	3.6	0	0
Oxidized acid %	0.15	7.71	20.4	2.47	0.87
Nitrogen %	trace	0.11	0.94	trace	trace

[1] Determined by the WIJS' method.
[2] Determined by the TAFFEL and REVIS[27] method, and expressed as cc. of 0.01 N-$Na_2S_2O_3$ per 1g oil.
[3] Expressed as red—grass number of the LOVIBOND tintometer.
[4] Determined by the FAHRION'S method[28]. See Chapter 2, Section 1.
[5] Determined by the KJELDAHL method.

As is seen in Table 1, pyrogallol-added oil is well protected from autoxidation, its color being not different from that at the start. On the other hand, the control oil without added pyrogallol did submit to autxidation and discolored into reddish-brown or reddish-orange.

Experiment 3.

Fresh shark liver oil was oxidized by letting air go through it at room temperature and every 20cc. sample oil was taken out in duplicate at intervals.

Table 2-*a*. Relations between the degree of oxidation and the color of oil developed on application of ammonia (shark liver oil).

Oxidized for (hr.)		0	16	40	91	140	768
Acid value	B	0.63	0.63	0.62	0.73	0.77	23.7
	C	0.60	0.59	0.60	0.71	0.70	16.2
Sapon. value	B	181.1	182.1	182.0	182.9	185.5	251.5
	C	182.0	183.7	181.3	182.7	183.1	221.5
Iodine value	B	139.7	138.1	137.7	136.8	135.4	61.47
	C	139.1	137.9	136.2	135.3	134.4	61.95
Color	B	R 1.1	1.1	1.3	1.3	1.2	0.9
		Y 20	21	20	20	11	7.3
		B 0.0	0.0	0.0	0.0	0.0	0.0
	C	R 3.9	2.0	3.8	3.9	6.0	dark red
		Y 20	20	20	20	20	
		B 0.0	0.0	0.0	0.0	0.0	

B; Treated with distilled water, C; Treated with conc. ammonia.

Table 2-*b*. Relations between the degree of oxidation and the color of oil developed on application of ammonia (shark liver oil).

Oxidized for (days)		0	7	19	30	35
Acid value	A	0.77	0.80	1.79	18.0	45.6
	B	0.74	0.75	1.54	13.5	30.3
	C	0.44	0.48	0.67	5.0	9.7
Sapon. value	A	180.7	181.5	187.2	223.2	240.3
	B	181.7	182.1	188.5	214.6	243.7
	C	183.3	184.8	188.7	196.1	202.4
Iodine value	A	123.2	122.9	112.8	89.50	72.26
	B	122.9	121.8	111.8	89.47	71.59
	C	121.2	123.1	113.4	89.31	71.90
Peroxide oxygen	A	1.09	1.14	3.35	11.14	43.18
	B	1.09	1.50	4.66	11.81	48.87
	C	1.09	1.50	4.01	—	—
Total nitrogen %	A	0.23	0.23	0.21	0.20	0.20
	B	0.23	0.22	0.20	—	—
	C	0.23	0.24	0.26	0.57	1.09
Oxidized acids %	A	0.23	0.25	1.03	6.97	15.15
	B	0.23	0.33	0.92	7.98	13.62
	C	0.24	1.01	1.43	8.44	25.34
KREIS-test	A	4.5	4.0	10.1	13.7	15
	B	3.2	3.6	6.4	9.6	12
	C	1.1	2.0	4.2	7.2	10
Color	A	R 0.0 Y 4.0 B 0.0	0.3 4.0 0.0	0.0 2.0 0.4	yellowish orange	yellowish orange
	B	R 0.1 Y 2.6 B 0.0	0.0 2.6 0.0	0.0 1.9 0.1	〃	〃
	C	R 0.5 Y 5.3 B 0.0	1.7 2.0 0.0	3.0 19.0 0.1	dark red	dark red
Note	A	—	—	—	liquid	semi-solid
	B	—	—	—	smells like fish-meal	
	C	—	—	—		

A: Control.

B, C: The same as before.

The samples were heated for 5 minutes at 90°C. immediately after mixing them with 5cc. of distilled water or 20% ammonia solution, respectively, while in the latter case the treated oil was further washed thoroughly with water until the washings became negative to the NESSLER's reagent. Then, the colors as well as the chemical characteristics of these samples were examined. Results are shown in Table 2 (*a* and *b*).

As seen in the tables, the oil discolors by no means even if met with ammonia so long as it remains not-oxidized, but if once oxidized it discolors in parallel with the extent of oxidation.

Section 2. The Second Factor

The author has pointed out in the preceding section that, although mere autoxidation of oil hardly brings about its discoloration, the first necessary condition for causing the discoloration to occur is nevertheless autoxidation. On the other hand, such substances as ammonia, cacein, and etc., which have an ability of discoloring the oil already autoxidized but not discolored, may be referred to the second factor.

Standing on this viewpoint, a number of factors which have been pointed out by many workers as having positive influence upon the discoloration phenomenon may well be classified into the following items, i.e., the first factor, the second factor, and so on.

As it has already been established that autoxidation is the first factor, the author treats in this section of the substances which have an ability of behaving as the second factor.

According to the result to be given in Exp. 4, a considerable number of basic, acidic, or neutral substances belonging to inorganic or organic field seem competent to be regarded as the second factor. As for the activity as the second factor, the general rule[7)8)] that the more strongly basic substance effects the stronger discoloration holds as it is among volatile basic nitrogen compounds too.

As already mentioned, fish-oil undergoes discoloration when kept in contact not with gelatin but with fish-meal or casein. So, at first sight, fish-meal and casein might be considered as the second factor. But the true reason why they react as the second factor is to be attributed to their usual contamination with ammonium salt, ultimately with ammonia. For, as is shown in Exp's 5 and 6, even active samples of fish-meal and casein turn out an inert substance like gelatin if their ammonium salt contamination is removed as completely as possible. Furthermore, the intensities of discoloration developed by various kinds of pure ammonium salts are virtually proportional to the strengths of their ammonia smell exhibited at room temperature, suggesting that free ammonia is the final form of the second factor in these experiments.

BEDFORD[1)] and OTTO[3)] are of opinion that the rusting of fish-products is induced by the propagation of bacteria, which is indissolubly tied to the formation of ammonia. Now, at least in the case of dehydrated or refrigerated fish-products, it will be certain that bacterial growth is stopped in effect; and yet even such products occasionally become discolored while stored. Then, how is to be explained this circumstance? The answer is that, as shown in the result of Exp. 6, presence of vital bacteria has no influence in itself upon the discoloration of fish oil. Accordingly, the author believes that, so long as dehydrated or refrigerated fish-products are concerned, what plays the role of the second factor is not their bacterial contamination but volatile basic nitrogen compounds contained in the raw fish; or, in other words, the most serious subject is the degree of freshness of the raw fish.

Experiment 4.

Each 5g of sardine-oil ((I) fresh oil, (II) oxidized oil prepared by heating I for 7 hours at 50°C., (III) oil obtained from the market), were treated with 0.1 mole of ammonia, mono-, di-, and tri-methylamine, respectively. Then, the color tinges of the treated oils were compared with one another by naked eye. The result is shown in Table 3 (*a* and *b*).

Table 3–*a*. Some characteristics of the sardine oils.

No.	Sample oil	Acid value	Sapon. value	Iodine value	Peroxide oxygen	Oxidized acids %	Color
I	fresh	3.8	192.5	182.3	trace	0	yellow
II	oil I oxidized for 7 hrs. at 50°C.	12.6	214.2	137.7	21.13	5.9	yellowish orange
III	from the market	5.5	199.6	158.0	12.81	1.9	orange

Table 3–*b*. Discolorations induced by volatile basic compounds.

No.*	Reaction at	NH_3	$NH_2 \cdot CH_3$	$NH \cdot (CH_3)_2$	$N \cdot (CH_3)_3$
I	room temp.	yellow	yellow	yellow	yellow
	100°C.	〃	〃	〃	〃
II	room temp.	reddish brown	reddish brown	dark reddish brown	dark reddish brown
	100°C.	dark reddish brown	dark reddish brown	brown	brown
III	room temp.	orange	reddish orange	reddish orange	reddish orange
	100°C.	reddish orange	〃	〃	〃

* The samples were the same as in Table 3–*a*.

A small quantity of crystals or aqueous solutions of various inorganic and organic compounds (see Tables 4 and 5) were mixed with each 5cc. of shark liver-oil and heated in a boiling water bath for 10 minutes. This liver oil had been oxidized beforehand with a current of air at 80°C. during 10 hours. The degree of discoloration attained was examined by naked eye. The sample of oxidized shark liver-oil which was treated merely with distilled water remained pale yellow without discoloring at all. The results are shown in Tables 4 and 5.

From these results it may be said that: Basic substances functions as the second factor more or less effectively, the efficiency increasing with the strength of basicity. The same relationship applies also to nitrogenous volatile bases. Not a few substances neutral or acidic in common sense also have to be taken into account as the second factor beside basic compounds; the problem remains open for a future study.

Table 4. Intensities of discoloration of autoxidized fish-oil induced by various inorganic compounds.

	K	Na	Ba	Ca	Mg	Al	Mn	Zn	Fe	Co	Ni	Sn	Pb	H	Cu	Hg	Ag
Metalic								###	##			##				+	###
Sulfide		+															
Bromide	+																
—ous Chloride	+	+	−	##	##	##	−	###	###	−		###	+	+	+	##	+
—ic Chloride								###	###						+	###	
Oxide					##	##		−	±				###				###
Sulphite		##															
—ous Sulphate	−	−	+	+	−	−	−	−	−		−		+	−	−		+
—ic Sulphate									###								
Nitrite		###															
—ous Nitrate	+	##	##	###	###	###		###	###	###	###			###		##	###
—ic Nitrate																###	
Carbonate	###	###	+	−									###				
Phosphate						+											
Hydroxide	###	###	###	###	−	+		+	+					−			
Acetate													−				
Citrate									−								

NO_2 gas ### ; $NaHSO_3$ ## ; $K_3Fe(CN)_6$ − ; $K_4Fe(CN)_6$ − ; K_2 or $Na_2Cr_2O_7$ − ; $KCr(SO_4)_2 \cdot 12H_2O$ − ; Nickel-ammonium-sulphate −.

Table 5. Efficiencies of various organic compounds discoloring autoxidized fish-oil.

1. No discoloration occurred : −
   betaine, *p*-acetotoluidide
2. Intensity of discoloration +
   glycine, leucine,, tyrosine, taurine, *l*-histidine-HCl, histamine, indol, sulfanilic acid, thiourea, diphenylamine, *m*-nitro-*p*-acetotoluidide.
3. Intensity of discoloration : ##
   arginine-HCl, asparagine, sodium glutamate pyridine, quinoline, ethylurethane, glucosamine-HCl, hydroxylamine, dimethylamine, phenylthiourea, N-N′-di-*o*-tolylthiourea, N-phenyl-N′-*o*-tolylthiourea.
4. Intensity of discoloration : ###
   trimethylamine, hypric acid, urea, naphthylamine, N-N′-diphenylthiourea, N-N′-di-*p*-tolylthiourea.
5. Intensity of discoloration : ###
   benzidine, anilin sulfate.

Experiment 5.

In this experiment, where the author intended to clarify the reason why fish-meal and casein play the second factor, and why gelatin does not, it was made conclusive that the reason lies in the difference as to whether there is or not any contamination with ammonium salt or ammonia.

§ Experimental procedure:

a) Detection of free ammonia.

Powdered samples of fish-meal, casein, and gelatin put respectively in a U-tube which was stoppered with cotton-wool at both ends were kept warmed in a water bath working at about 50°C. A current of air freed from basic gases by washing with dilute H_2SO_4 was led through the U tube into a solution of NESSLER reagent contained in a test tube. After 60 minutes aeration, free ammonia was confirmed of its presence or absence according to whether a positive NESSLER reaction came to appearance or not.

b) Qualitative and quantitative determination of ammonium salt.

Ammonium salt was thoroughly extracted from the samples by boiling the latter in a dilute solution of hydrochloric acid. Qualitative test was done with NESSLER reagent on this extract after neutralization. In quantitative determination, trichloroacetic acid was first added to the above extract so that dissolved protein might be filtered off, and on an aliquot of the filtrate the ammonia was estimated by the KJELDAHL method.

c) Relative intensities of ammonium salts as the second factor.

Unit gram of crystal or 1cc. of a concentrated solution of ammonium salt was added to every 5cc. of shark liver-oil which had beforehand been oxidized with an air current driven through it at 80°C. for 10 hours. The oxidized oil remained pale yellow. It was further heated in a boiling bath for 30 minutes. After that the degrees of discoloration of the different samples were compared with one another by the unaided eye.

§ Sample used:

a) Fish-meal: Prepared from sardine flesh in this laboratory.

b) Casein & Gelatin: E.P. reagent, used after washing thoroughly with ether.

§ Result obtained:

As shown in Table 6, free ammonia was detected only for the sample of fish-meal but not for that of casein or gelatin. Ammonium salt, on the other hand, was present in considerable amounts in the fish-meal as well as the casein, while it could not be detected of its presence in the gelatin sample. This result seems to corroborate the author's supposition given above regarding the difference in activity of these materials as the second factor.

Table 6. Forms of ammonia in the proteinous samples

Sample	Free ammonia	Ammonium salt
Sardine meal	+	##
Caseine	−	##
Gelatine	−	−

Table 7. Relation between the time of heating at 150°C. and NH_3—N content of casein.

Heating minutes	NH_3—N mg. %	Heating minutes	NH_3—N mg. %
0	181	80	47.7
20	123	160	12.1
40	90.3		

The amount of ammonium nitrogen determined on the casein sample was 181 mg%, and after heating the sample for 160 minutes at 150°C. the value was reduced to only 12.1 mg% as shown in Table 7, suggesting that the contaminative ammonium salts decompose on heating rather promptly.

The intensities of several different ammonium salts as the second factor were found to be arranged in the following order:
Carbonate, Nitrate>Secondary phosphate>Chloride>Oxalate>Sulphate.

Experiment 6.

This experiment aimed to verify that the second factor action of the casein is due to the impurities like ammonium salt, but not to the bacterial contamination.
§ Experimental procedure:

Each 5g of casein sample were separately put into cotten stoppered 30 cc. ERLEMEYER flasks, which had been sterilized in a usual way. To them each 30 cc. of fresh shark liver-oil were added though a BECKFELD-N bacteria filter, and the mixtures were kept for two weeks at 37°C. After that bacteria were cultured in aerobic as well as anaerobic condition using small parts of the mixtures. Oil was then extracted from the remaining parts with ether, and the colors of thus extracted oils were noted.
§ Samples used:

The samples of casein here employed were as follows:

a) E.P. grade market casein containing 180 mg% of ammonia nitrogen.

b) A specimen prepared by heating E.P. grade casein for 40 minutes at 150°C. so that ammonia nitrogen content decreased down to 90 mg%.

c) E.P. grade casein further purified by boiling and washing in dilute HCl until the washings became negative to NESSLER's reagent and then dried up in an electric oven.

§ Result obtained:

As shown in Table 8, the larger the amount of contaminative ammonium salt, the stronger discoloration took place. Furthermore, with the casein freed from ammonium salt contamination discoloration did not occur at all. Accordingly, it may be concluded that the action of casein as a second factor is nothing but the action of ammonium salt usually present as an impurity.

Table 8. Relations between the rusting of fish-oil and the existence of bacteria.

Casein used	Color of fish-oil	Existence of bacteria and its form
a*	brown	purely cultured spore forming large rod bacteria****
b**	orange	〃
c***	pale yellow	〃

a* : Market casein ; b** : a* was heated 40 min. at 150°C.
c*** : a* was washed thoroughly with dilt. HCl and next with aq. dest. until the washings became negative to NESSLAR reagent.
**** : This bacteria had existed already in the market casein before the experiment.

Now, there will naturally be no objection in extending this conclusion to the case of fish-meal.

The influence of bacterial contamination upon the rate of discoloration seems to be out of the question at least in the present experimental condition. For, in this experiment the achieved discolorations are widely scattered among different samples notwithstanding that the samples are contaminated equivalently with the same sort of bacteria.

Section 3. The Constituents of Oil Having Relation to the Discoloration

It has been reported that the yellowing of white paint proceeds in proportion to the degree of unsaturation of the basal oil, and also supposed that "oxidized acids," the essential colored fraction of discolored oil, may have been derived from fatty acids of higher unsaturation[8)18)22)29)30)]. In these papers, however, no comment has been made as to whether or not other constituents of oil such as fatty acids of lower unsaturation, saturated fatty acids, glycerine, and unsaponifiable matters bear any relation to the discoloration phenomenon of oil.

In this section, studying the above circumstances, the author arrives at the conclusion that, whereas in the natural discoloration the essential colored substance may likely be derived mainly from rather highly unsaturated fatty acids, in vitro not only fatty acids of lower unsaturation but also saturated fatty acids are possible to produce a colored substance if they contain carbon atoms over twelve. Glycerine, though it develops only a much weaker color as compared with fatty acids, has some relation to the discoloration. Unsaponifiable matters bear no relation to the occurrence of discoloration.

Experiment 7.

In this experiment the author intends to know whether a highly unsaturated fatty acid is the unique component of oil that has an intimate relation to the discoloration phenomenon.

Table 9. The natures of the separated fatty acids from shark-liver oil by HILDITCH's method.

		Acid value	Iodine value	Peroxide oxygen	Oxidized acids %	KRIES test red value	Yield gram	Note
Sample oil		1.39	127.0	3.59	4.01	3.0	100	
Liquid fatty acids		140.6	153.0	5.77	0.32	3.0	57	reddish orange
Solid fatty acids	F-1	206.1	26.74	2.84	nil	0	5.0	white
	F-2	200.6	50.95	7.37	trace	0.4	11	greyish yellow
	F-3	192.3	104.5	5.19	trace	3.3	15	dark orange
Unsaponifiable matters		—	—	—	—	—	3	

§ Experimental procedure and result obtained:

Aburazame liver-oil was saponified in a usual way and unsaponifiable matter was separated completely off from fatty acid fraction. The fatty acid fraciion was submitted to the alcohol-lead salt method[31] so that solid- and liquid-fatty acid might be separated from each other. The solid fatty acid fraction was further fractionated into three subfractions by recrystallization from its 94% alcoholic solution. The chemical properties as examined of these sub-fractions are given in Table 9.

In addition to the above subfractions, freshly distilled glycerine was also used in the discoloration test. Each of them was heated at 90°C. under sending ammonia gas into it. On such treatment, color of the liquid fatty acid fraction changed to dark reddish brown instantaneously, while that of the solid fatty acid subfractions changed to dark red, reddish orange, and orange respectively according to the order of their decreasing iodine value.

Although the discoloration recorded in Table 9 is weakest for the fraction F-1, the fraction with least iodine value, even the very fraction discolored to chocolate brown if it had previously been oxidized by keeping it aired for about one hour at 90°C. or so.

On the other hand, while the unsaponifiable fraction that had been oxidized with air did not become discolored on the subsequent application of ammonia, glycerine did become stained on the same treatment as above eventually to light orange, the color intensity, however, remaining far much weaker than that of fatty acids.

Experiment 8.

In connection with Exp. 7, this time the author intends to know whether or not the saturated fatty acid components cause the discoloration of oil.

§ Experimental procedure:

4 g of fatty acid sample was first oxidized by airing the sample at 90°C. for an hour at the rate of 25 *l* per hour. After that ammonia gas was introduced during 20 minutes at the said temperature. The color developed was noted.

§ Sample used:

As samples of linearly chained saturated fatty acids, acetic acid homologues up to palmitic acid were employed. The individual acids were purified in the form of methylester by fractional distillation at the theoretical boiling points of these esters prior to their preparation. When further purification was demanded, recrystallization from alcoholic solution was carried out on a suitable salt of the acid.

For the purpose of comparison a purified preparation of oleic acid was as a standard submitted to the same test. The characteristics of the sample fatty acids were as follows, the corresponding referential values being cited in parentheses:

a) Acetic acid, C-2. M.P.: 17°(16.7)[30]; B.P.: 118°(118.1)[33]; N.V.: 934.6(934.52)
b) Propionic acid, C-3. B.P.: 141°(141)[33]; N.V.: 758.5(757.8)
c) *n*-Butyric acid, C-4. B.P.: 162°(162.4)[33]; N.V.: 638.3(637.05)

d) *n*-Valeric acid, C-5. B.P.: 86–88°/15mm. (86–88/15mm)[33]; N.V.: 549.8 (549.57)
e) Caproic acid, C-6. B.P.: 106–108/16mm. (107.3/16mm)[33]; N.V. 483.5(483.28)
f) Caprylic acid, C-8. M.P.: 16°(16–16.5)[32]; B.P.: 134–136°/16mm(134.6/16mm)[33] N.V.: 389.9(389.23)
g) Capric acid, C-10. M.P.: 31°(31.5)[32]; B.P.: 144–146°/8mm(145.5/8mm)[33]; N.V.: 326.0(325.86)
h) Lauric acid, C-12. M.P.: 43.7°(43.6)[32]; B.P.: 141–143°/2mm(141.8/2mm)[33]; N.V.: 280(280.23)
i) Myristic acid, C-14. M.P.: 554°(53.8)[32]; B.P.: 160–162°/2mm(161–162°/2mm[33] N.V.: 245.9(245.81)
j) Palmitic acid, C-16. M.P.: 63.2°(62.5)[32]; B.P.: 178°/2mm(179/2mm)[33]; N.V.: 218.8(218.92)
k) Oleic acid, C-18. N.V.: 197.97(198.75); I.V.: 90.14(90.07)

§ Result obtained:

As shown in Table 10, oleic acid causes indeed an intense discoloration, and the fatty acids having more than twelve carbon atoms suffer discoloration the more intensly with increasing chain length, but never do so such having carbon atoms below ten.

Table 10. The degree of discoloration of saturated *n*-fatty acids treated with molecular oxygen and subsequently with gaseous ammonia.

Fatty acids	Degree of discoloration	Note
Acetic acid	−	
Propionic acid	−	
n-Butyric acid	−	
n-Valeric acid	−	
Caproic acid	−	
Caprylic acid	−	
Capric acid	−	
Lauric acid	+	faint orange yellow
Myristic acid	++	orange
Palmitic acid	+++	dark brown
Oleic acid	+++	dark brown

Remarks on the Function of the Second Factor

It is somewhat difficult to infer the mode of interaction between the second factor and oxidized oils, because, as already mentioned in section 2, not only basic substances but also the so-called neutral and acidic substances can be enumerated in certain cases as the second factor.

The function of the second factor, however, may be considered for the time being from two different points of view as follows:

1) Chemical combination with the oxidized oil, giving rise to a colored substance.

2) Catalysis in spontaneous formation of a colored substance from oxidized oils.

Supposing that the function of the second factor belongs to the case 1), the BREE's law may be applicable to the reaction system so long as the reaction mixture is of an extreme dilution. Thus, if the concentrations of a second factor instead of the color intensities attained are calibrated on abscissa, and the corresponding optical density of the reaction system on ordinate, a relation expressible by a straight line would be obtained.

However, as it has been obtained actually a relation to be represented rather by a curve instead of a straight line*, it might be said that, at least in the case of ammonia, the function of the second factor partaking in the discoloration of oil is not to produce its compound with some constituent of the oil, but to behave merely as a catalyst.

CHAPTER II. STUDIES OF THE OXIDIZED ACIDS

Section 1. Oxidized Acids as an Essential Coloring Component of Discolored Oil

Occasionally, this type and that of atomic group were presumed to be present in the discolored oil as its chromophore[14)18)29)34)35)]. A trial to fractionate the discolored oil, on the other hand, was carried out by FAHRION[28)] as early as in 1891, and repeated subsequently by some researchers, i.e., MUNZERT[8)], and TSUJIMOTO[22)30)], all falling in with FAHRION. To verify the entity of the color of the discolored oil, therefore, this FAHRION's colored fraction, the so-called "oxidized acids," is thought to be the most likely place to find it.

Now, as it is not very easy to find original papers treating "oxidized acids," it will surely aid readers in appreciating the definition of the term and also the point of the author's argument to reproduce here the FAHRION's procedure rather in detail. It says:

According to the degrees of oxidation, 2 to 5 g of the sample are saponified in the usual manner with alcoholic potash; the alcohol is evaporated off, the soap is dissolved in hot water, transferred to a separating funnel, and decomposed with hydrochloric acid. After cooling, the liquid is shaken with petroleum ether of boiling point below 80°C., and allowed to stand until it has separated completly into two clear layers. The insoluble oxidized fatty acids will be found to adhere to the side of the funnel or forming a sediment in the petroleum ether layer. The aqueous layer is drawn off, the petroleum ether layer is poured off, if necessary through a filter, and the oxidized acids are washed with petroleum ether to remove residual fatty acids. In case the amount of oxidized fatty acids be large, it is advisable to dissolve them in alkali, decompose the soap with hydrochloric acid, and shake up again with petroleum ether in order to remove completely any occluded soluble fatty acid. For the quantitative determination the oxidized acids are dissolved in warm alcohol, the alcohol is evaporated off, and the residue

* unpublished

dried until the weight remains constant. Thus the proportion of oxidized acids is found.

Now, if it is true that the oxidized acids are an essential coloring fraction of a discolored oil, the part of the discolored oil other than oxidized acids should be as light in color as the fresh oil. And furthermore, at least for samples originating from the same oil, there should exist an intimate relation between their content of oxidized acids and degree of discoloration.

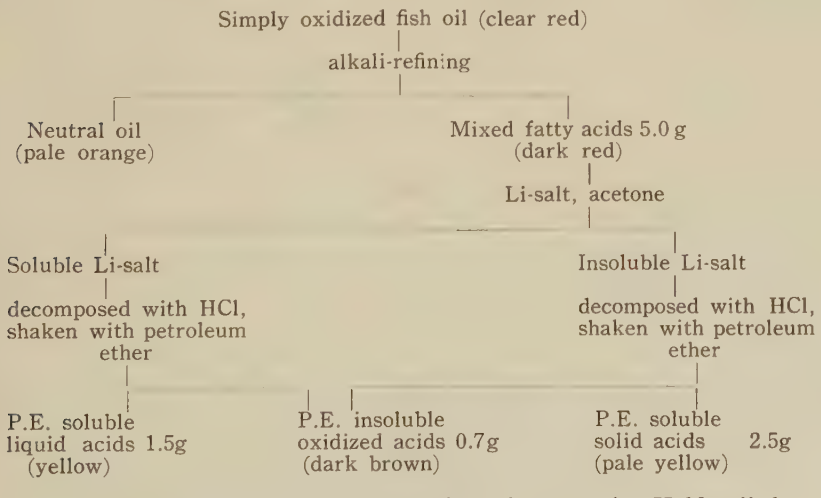
In this point of view, the author separated the oxidized acids from discolored oils after the FAHRION's method (see Exp. 9), and confirmed that the remainder of the oils was not discolored in any case. In addition, it was also found that a pretty good parallelism existed between the oxidized acids content and the degree of discoloration, as shown in Table 13 for Exp. 10. These results seem to be an evidence of that the oxidized acid is an essential coloring component of the discolored oil.

Experiment 9.

1) *Hokke*-oil, on the market, which had discolored red, was submitted to the usual alkali refining. In this case, the neutral oil fraction became decolorized almost perfectly, while the free fatty acid fraction remained dark red. Complementary halves consisting respectively of mixed fatty acids of higher- and lower-unsaturation, into which the free fatty acid fraction was divided by the lithium salt-acetone method, were colored dark red.

But after oxidized acids had been separated away from these fractions by treatment with petroleum ether, the remainder of both the fractions appeared decolorized to pale yellow, while the isolated oxidized acids were dark brown in color. This state of affairs will be seen in Table 11.

Table 11. Decolorization of oxidized fish oil (taken from *Pleurogrammus azonus* J. and M.) by alkali-refining.

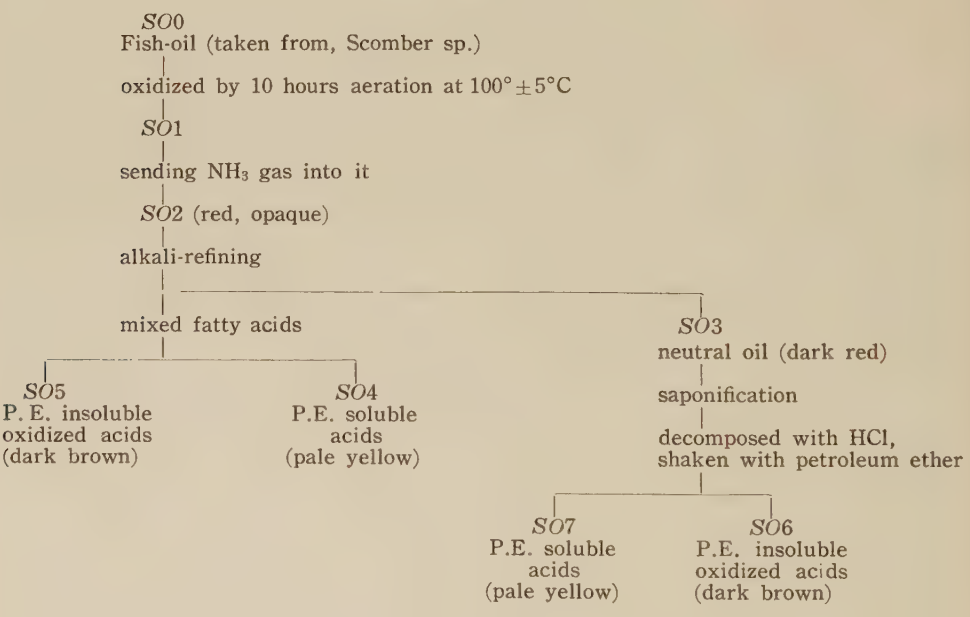

Simply oxidized fish oil (clear red)
alkali-refining
Neutral oil (pale orange)
Mixed fatty acids 5.0 g (dark red)
Li-salt, acetone
Soluble Li-salt
decomposed with HCl, shaken with petroleum ether
Insoluble Li-salt
decomposed with HCl, shaken with petroleum ether
P.E. soluble liquid acids 1.5g (yellow)
P.E. insoluble oxidized acids 0.7g (dark brown)
P.E. soluble solid acids 2.5g (pale yellow)

It may well be accepted in this case that the sample *Hokke*-oil has been

discolored, as is mostly the case, by reason of its inclusion of free oxidized acids, and the oxidized acids in turn are distributed widely among fatty acids of various degrees of unsaturation.

2) Fresh mackerel oil was oxidized by aeration and made to discolor with ammonia gas. The opaque red oil thus obtained was submitted to alkali refining as in the case of 1). The neutral oil fraction here separated was found decolorized to some extent but still retained a red tinge. When this fraction was saponified and deprived of oxidized acids by the FAHRION's method, however, the remainder was found to have been decolorized to pale yellow. This remainder, the free acid fraction of the sample oil, was further divided into two fractions, i.e., petroleum ether-soluble pale yellow fatty acids and petroleum ether-insoluble dark brown oxidized acids. The circumstances are noted in Table 12. From these results it can be inferred that in the sample oil the oxidized acids exist not

Table 12.



only as free acids but also in the form of glycerides.

3) In the course of usual saponification with alcoholic potash, the solution of autoxidized oil becomes colored spontaneously dark red to brown; and it is usual that the stronger oxidation the oil submitted to, the more deeply colored the saponified solution.

Recently, it was suggested by PALLAUF[37] that a photometric examination of a saponified solution is useful for estimating the rancidity. Thus, if a saponified solution is colored deeply, one is safe to say of the presence of oxidized acids but is not if the solution is only pale in color.

Referring to the above fact, one may well assume the following situation: The oxidized acids component of oxidized oil is inherently colorless, and

undergos discoloration first when acted by the second factor.

When the action of the second factor is only a mild one as in the case of 1) or 2), the oxidized acids can exist in the form of glyceride as well as in that of free acid. But if the action is very strong as in the case of 3), there occurs discoloration of the colorless oxidized acids simultaneously with the breakdown of glyceride linkages.

Experiment 10.

§ Experimetal procedure:

Fresh samples of shark liver-oil were oxidized respectively in various ways and 5 cc. of each sample were mixed with 5 cc. of concentrated aqueous ammonia and heated for 5 minutes on a boiling water bath. The samples thus treated were washed thoroughly with water until the washings became negative to NESSLER's reagent. After that the oil was dried in a usual manner on anhydrous sodium sulphate and the color as well as the oxidized acids content was determined.

Table 13. Relations between the color of the saponified matter, the contents of oxidized acids and the red tinge developed on treatment with ammonia.

No.	Acid value	Sapon. value	Iodine value	Peroxide oxygen	Oxidized acids %	Color of the saponified matter	*1 NH_3	Remarks
501	0.55	166.3	125.6	0.22	trace	pale arrange	−	original oil
502	2.42	165.8	85.41	0.21	〃	pale orange	−	*2
503	1.35	186.6	109.3	3.07	4.52	brown, opaque	##	*3
504	21.16	191.0	58.06	32.8	10.21	〃	##	oxidized 30 days in a light place
505	2.85	213.3	116.6	2.36	2.41	〃	#	oxidized 30 days in a dark place
506	0.66	170.4	122.7	0.13	0.59	pale brown	−	oxidized with dilt. H_2SO_4 and $KMnO_4$
507	0.43	183.1	119.8	6.98	0.85	dark red	+	
508	1.36	185.5	115.0	3.20	1.15	dark brown	+	
509	1.89	176.8	105.6	1.45	2.15	〃	#	
510	4.64	170.4	82.79	2.40	3.14	〃	#	oxidized with BECKMANN's mixture
511	4.74	180.4	108.9	2.58	1.24	dark red	+	
512	0.24	179.7	121.5	3.93	trace	pale brown	−	oxidized with 30% H_2O_2
513	0.41	164.9	118.4	2.81	〃	〃	−	
514	1.94	180.6	123.8	3.77	0.41	reddish orange	+	oxidized with O_3

Note : *1 Degree of the reddish color of oil which is induced by conc. NH_4OH (5g of oil and 5cc of conc. NH_4OH were mixed and heated 5 mim. on a water-bath),
*2 Polymerized at 270°C., 120 min. by sending CO_2 into the oil.
*3 Oxidized at 130°C., by 240 min. aeration.

The said ways of oxidizing the samples were as follows:

a) Exposure to the air in dispersed light for 30 days at room temperature.
b) Exposure to the air in a dark place for 30 days at room temperature.
c) Oxidation with the potassium permanganate and dilute H_2SO_4.
d) Oxidation with the BECKMANN's mixture.
e) Oxidation with 30% hydrogen peroxide.
f) Oxidation with ozone at room temperature.
g) Oxidation by aeration for 240 minutes at 130°C.
h) Heating for 120 minutes at 270°C. in carbon dioxide atmosphere.

§ Results obtained:

As is shown in Table 13, it may be recognized that an intimate relation exists between the oxidized acids content and the degree of the discoloration caused by the action of ammonia.

Section 2. Chemical Characteristics of the Oxidized Acids

As it has been confirmed in the preceding section that the oxidized acids are an essential coloring component of discolored oil, the author will use this fraction hereafter as the samples in the study of the nature of colored substance, which will be described below.

In entering into carrying out this study, it seem necessary first to know the chemical characteristics of the oxidized acids, but there have been offered hitherto only a few informations except the LEWKOWITSCH's[38] description.

In this section, therefore, the author attempts to know some chemical characteristics of the oxidized acids of various origins, following LEWKOWITSCH.

Experiment 11.

§ Experimental procedure:

Neutralization value, saponification value, iodine value, total nitrogen, and average molecular weight were determined for oxidized acids of various origins. Iodine value determination was made by the WIJS's method, and total nitrogen by the KJELDAHL. Oxidized acids were found to dissolve completely in camphor but were not to do so in *m*-dinitrobenzene, phenanthrene, and bornylchloride. So the average molecular weight determination was made by the RAST method, using camphor as solvent.

As LEWKOWITSCH had already identified a lactone linkage in the oxidized acids of linoxin, the author inquired in the first place into the problem whether such a linkage was present also in those of fish-oil. This problem was answered by measuring the difference between saponification- and neutralization-value after alternatively repeating saponification and acidification.

§ Sample used:

The following eight kinds of oxidized acids were used as the sample:

S-1) & K-1). The samples were obtained respectively from mackerel- and bonito-oil, both having been oxidized but not discolored.

S-2) & K-2). The samples were prepared from discolored mackerel- and

bonito-oil, respectively, in the following manner: The same raw oils as those used for S-1) and K-1) were first made to discolor with ammonia gas. The discolored oils were washed thoroughly with water and dried on anhydrous sodium sulphate, after which the oxidized acids fraction was taken out. The fraction was then dissolved in a small quantity of acetone and submitted to dissolution and precipitation repeated alternatively with large quantities of petroleum ether and with water. This enabled all contaminations to be removed.

W-1) & W-2). The samples were obtained from two kinds of whale oil which had been offered for the market and discolored red to appearance.

L-1). The sample was prepared by extraction from a gelatinous film of dried linseed oil.

H-1). The raw material was mixed fatty acids for sale. It had been derived from *Hokke*-oil and discolored reddish brown. The sample was obtained by precipitation form a petroleum ether solution of this commodity.

§ Results and discussion:

Results of the present experiment are summarized in Tables 14 and 15.

From these tables it will be seen that these oxidized acids differ in their characteristics considerably from each other not only when they were different

Table 14. Characteristics of oxidized acids isolated from various oils.

Oxidized acids		Acid value	Sapon. value	Iodine value	Total-N %	Molecular wt. RAST's method
Scomber oil	S-1	171.4	296.3	110.6	0.08	—
	S-2	199.7	253.4	107.0	0.69	507.6
Bonito oil	K-1	132.0	212.4	139.7	trace	548.9
	K-2	165.6	261.2	151.4	0.41	554.4
Whale oil	W-1	195.3	200.5	61.93	2.96	1143
	W-2	151.8	224.3	73.15	1.55	1004
Linseed oil	L-1	—	—	—	0.21	679.5
Mixed fat acid of Hokke* oil	H-1	158.7	250.7	85.85	0.12	665

S-1, K-1 : Isolated directly from the correspondent autoxidized oils.
S-2, K-2 : Autoxidized oils by applying ammonia and washed with water until NÉSSLER reaction became negative. The final oxidized acids were isolated from the discolored products.
W-1, W-2 : Isolated from market whale oil, discolored red, and used without refining.
* : *Pleurogrammus azonus* J. and M.

Table 15. Effect of repeated saponification upon the characteristics of oxidized acids (from sardin oil).

Number of repeat	Neut. value	Sapon. value	Iodine value	Total nitrogen	Mol. wt. as calculated from		
					N. V.	S. V.	RAST
1	181.4	225.9	79.88	trace	309.3	248.4	696.1
2	194.8	223.1	82.81	〃	288.0	251.5	695.1
3	192.9	222.9	82.40	〃	290.8	251.7	674.5

as to the kind of raw material but also when they were the same in kind but variant in the history before isolation.

Hereof, it must not be overlooked the fact that a marked difference prevails among the different samples of oxidized acids as regards their nitrogen content —a criterion for the catalytic share of the ammonia. Thus the measured nitrogen content fluctuates from a trace to 2.96%.

Some researchers[3)4)5)] are of opinion that there may exist an intrinsic relation between the occurrence of discoloration and the presence of nitrogen in the discolored oil or the oxidized acids. The present author, however, conceives that the nitrogen in oxidized acids may be no normal element but a sort of impurity. The reasons are as follows: 1) As mentioned in the preceding section, the optical density of discolored oil does not increase in proportion to the amount of admixed ammonia. 2) Although several types of ammonium compound may have been formed by the action of gaseous ammonia, it is certein that ammonium salt, amide, and aldehydeammonia decompose without fail in the course of the separation of oxidized acids. ODA et al[39)]., say that liquid ammonia does not react upon the double bond of unsaturated fatty acids. It seems hence, to be the case also with gaseous ammonia. Any more types of ammonia derivatives other than those above mentioned are not possible to be conceived of their formation so long as gaseous ammonia is concerned. 3) During the repeated precipitation of oxidized acids dissolved in alcohol or acetone with large quantities of water, the nitrogen content decreases step by step; nevertheless there occurs no visible depression of the color intensity. 4) Resinous nature of oxidized acids render the removal of ammonia inefficient.

LEWOKWITSCH has pointed out the relatively high acetyl value and the existence of both unsaturated- and lactone-linkages as regards the characteristics of oxidized acids. From Tables 14 and 15 the existence of both unsaturated- and lactone-linkages is recognized. It is supposed that most of these lactone linkages in the oxidized acids may be of γ- or δ-type, because, as shown in Table 15, the difference between saponification- and neutralization- values decreases only a little even through an exhaustive saponification and acidification.

As for the relatively high acetyl value of the oxidized acids the author will make some comments later on.

Section 3. Fractionation of Oxidized Acids

Since the existing oxidized acids have been prepared by such a rough method as described in the preceding section, it may be apprehended that these oxidized acids still consist of various species of oxidation products of fatty acids. These petroleum ether-insoluble products might have such polar groups as –OH, –CHO, and>CO in their chemical constructions.

Though it is possible that there partly exist some inherently colored matters such as 9, 10-diketostearic acid, or some other substances that form a colored matter through the action of alcoholic potash, most of the said products are expected to be colorless not only in their inherence but after the application of lcoholic potash. All things considered, it seems not too much to say that the

ultimate ringleader of the discoloration phenomenon still remains to be caught.

In the present section, therefore, the author intends to fractionate the oxidized acids which is obtained from pure oleic acid, the starting material, with the purpose of grasping the chemical entity of the coloring matter. The reasons why oleic acid was used as starting material are as follows: 1) Oleic acid is a usual component of oil. 2) Pure sample of it is obtainable with ease. 3) Its oxidation products have been well studied.

Experiment 12

§ Experimental procedure and results obtained

The oxidized acids used in the present study were prepared according to the routine procedure from oleic acid which has been aired for 50 hours at 100°C. The yeild of the oxidized acids was about 40 per cent.

7 g of the oxidized acids, colored brownish orange, was mixed with 100 cc. of chloroform. After digesting the mixture for 10 minutes on a boiling water bath, it was cooled down to room temperature and allowed to stand over night in a refrigerator at −5°C. The precipitate here formed was separated by quick filtration while cold, 1.4 g of greyish white fine crystals being obtained. The crystals were further submitted to repeated recrystallization from 90% alcohol, and there obtained 0.16 g of white lustered fine scales, m.p. 131–132°C. Any depression of melting point could not be detected on a mixture of this sample and an authentic preparation of 9, 10–dihydroxystearic acid of m.p. 132°C. Neutralization value was 177.7 (theoretical: 177.4). The preparation was decided as trans–9, 10–dihydroxystearic acid. After the removal of alcohol from the collective mother liquor of the above recrystallization, the residue in turn was submitted to repeated recrystallization from 60% alcohol; 0.96 g of white fine crystals, m.p. 93.5°C., were obtained. The melting point of a mixture of this preparation with authentic 9, 10–dihydroxystearic acid of m.p. 95°C., was 93.5–95°C. Neutralization value was 177.5. This preparation was decided as cis–9, 10–dihydroxystearic acid. Alcohol was evaporated off from the collective mother liquor of the above second recrystallization, and the residue was submitted to the recrystallization this time from 40% alcohol, and a small quantity of white fine crystals was obtained. The melting point of these crystals mixed with authentic 10–hydroxystearic acid of m.p. 84°C., was 83.5–84°C. This preparation was identified to be 10–hydroxystearic acid.

On the other hand, chloroform was evaporated off from the filtrate separated from the abovementiond greyish white precipitate of hydroxy acids. The residue was submitted to further fractionation into carbonyl- and noncarbonyl-fractions. This was carried out by the following procedure: 2 g of the residue was dissolved in 50 g of absolute methanol and then were added 5 g of gracial acetic acid and 4 g of Girard–P reagent[40].

The mixture was boiled for one hour and then poured into 250 cc. of ice-water alkalinized with 6 g of sodium carbonate. Non-carbonyl fraction was extracted with ether from this ice-water mixture as a fraction colored orange yellow. Yield was 1.6 g. To the residue of the ether extraction was added concentrated

sulphuric acid till its concentration attains to about 2 N. The acidified solution was transferred into a separating funnel and added with ether. After being kept standing over night the funnel was shaken vigrously. The ether layer was separated subsequently, washed with water, and then dried over anhydrous sodium sulphate. After the ether was evaporated off, 0.28 g of carbonyl fraction was obtained as a reddish brown viscous liquid.

Some characteristics measured for intact oleic acid, oxidized oleic acid, and noncarbonyl fraction of the oxidized acids are recorded in Table 16.

Table 16. Properties of intact oleic acid, oxidized oleic acids prepared by 50 hours aeration at 100°C., and noncarbonyl fraction contained in the oxidized acids isolated from the aerated oleic acid.

	Acid value	Sapon. value	Iodine value	Peroxide oxygen*1	Oxidized acids %	Hydroxy value	mol. wt. (RAST)*2
Intact oleic acid	199.3	0	89.87	0	0	—	—
Aerated oleic acid	159.6	283.1	19.94	4.05	40.0	56.6	378.6
Non-carbonyl fraction in oxidized acids	134.3	240.1	23.31	—	—	127.5	411.8

*1 0.01N $Na_2S_2O_3$ in cc., per 1 g oil.

*2 Camphor as solvent.

Results of qualitative detection of aldehyde, ketone, acid-anhydride, ester, and lactone, which was performed both on the carbonyl- and noncarbonyl fractions of the oxidized acids by GRAF's[41] method are gived in Table 17. As for the carbonyl fraction, furthermore, its inclusion of aldehyde acetal or polymerized aldehyde was confirmed.

Table 17. GRAF's-test for carbonyl and noncarbonyl fraction in the oxidized acids isolated from the aerated oleic acid.

	Ketone	Aldehyde	Acid anhydride	Ester or Lactone
Noncarbony fraction	−	−	−	+
Carbonyl fraction	+	−	+	−

Now, it is seen that 0.12 g of sample was lost in the course of the present fractionation. Then, assuming that the composition of the starting-sample is inferable from the proportion of the component fractions actually obtained, the amounts of carbonyl- and noncarbonyl-components having been contained in the chloroform mother liquor may be calculated as 0.83 g and 4.77 g, respectively. Accordingly, the composition of the oxidized acids sample is inferred to be: hydroxy acids (colorless): 20%, noncarbonyl compounds (orange yellow): 68.1%, carbonyl compounds (darkly colored): 11.9%.. It is noteworthy, indeed, that a portion amounting to 90% of the brownish orange preparation of oxidized acids is of rather a faint color.

SKELLON[42] has oxidized a highly purified sample of oleic acid by treating

it with a current of dry oxygen for 260 hours at 100°C. without any catalyst applied. From the oxidation product there obtained he has isolated 15% as 9, 10-dihydroxystearic acid of m.p. 131–132°C., and 10% as C_{18}-hydroxy acid. He has also isolated, from an oleic acid sample which had been oxidized by introducing a dry oxygen current for 12 hours at 120°C. in the presence of a trace of blown whale oil as catalyst, 16–19% as dihydroxy stearic acid of m.p. 131°C., 6–7% as dihydroxy stearic acid of m.p. 95°C., and 5–6% as 10-hydroxy stearic acid, m.p. 84°C.

Though the present experimental condition is correspondent to that of the SKELLON's noncatalytic oxidation, the oxidation products closely resemble rather those of catalytic oxidation obtained by the same author.

In any case, it may be said that the dark color of so-called oxidized acids is attributable to a carbonyl component present in only a small proportion.

Section 4. Correlation of the Oxidized Acids to the Drying-Phenomenon of Oil

According to the author's experience, it is usual that the more viscous the oil becomes in the course of oxidation, the larger amount of oxidized acids are formed. Although, as mentioned in the preceding section, the oxidized acids prepared by the FAHRION's method are a darkly colored substance, they are likely to remain as a colorless material at least till the autoxidized oil meets with the second factor, to which is to belong of course the alcoholic potash used in the said method.

In this section the author intends to inquire into the correlation of the colorless- or leuco-oxidized acids to the drying-phenomenon of oil. But, since the isolation of oxidized acids is quite difficult to be carried out without any influence of the second factor so as to give a colorless preparation, the discussions that follow will be advanced under an assumption that no appreciable weight change attends the discoloration of the leuco-acids.

Experiment 13.

1. Difference in the oxidized acids content between the liquid phase of fish oil and the drying surface film formed on it.

§ Experimental procedure and result obtained:

A soft dry film of *Hokke*-oil was compressed between the layers of filter paper to remove liquid oil as completely as possible. (This stock *Hokke*-oil was that which had been refined by means of alkali so as to become light orange in color, even though it smelt still somewhat rancid, and kept stored in a stoppered brown glass bottle over an year until the time of experiment.)

Saponification value and oxidized acids content determined of both this pressed film and the liquid phase of the stock oil are shown in Table 18.

As shown in the table, a far much oxidized acids are present in the dry oil film as compared with the liquid phase,

Table 18. Oxidized acids content in the bluk of autoxidized fish* oil and in the film formed on its surface.

		Saponification value	Oxidized acids (%)**
F	surface film	225.6***	23.44
L	liquid phase	319.4	1.27

* : *Pleurogrammus azonus* J. and M.
** : After FAHRION method.
*** : This value seems to be somewhat lower than the true one, on account of incomplete saponification, for, in evaporating-off the alcohol from the saponified mixture, some droplets of oil appeared floating, which disappeared on adding a few pieces of KOH.

2. Fractionation of a viscous autoxidized oil by means of a solvent and examination of chemical properties of the fractions isolated.

§ Experimental procedure and result obtained:

23 g of viscous, pale yellow Saury pike-oil, oxidized acids content of which was 9.55%, was aerated for 15 hours at 55°C. and dissolved subsequently in 55 cc. petroleum ether (b.p. 30-60°C.). The solution thus obtained was separated into three fractions by stepwise adding 97% alcohol. The yields and a few chemical properties of these fractions are given in Tables 19 and 20.

Table 19. Fractionation of viscous autoxidized *Samma** oil, of which 23 gram was dissolved in 55 cc. of petroleum ether and added with various amounts of 97% alcohol.

	Lower layer separated	Yield (g)	Appearance
Fraction I	on first addition of 64 cc. alcohol	8.5	orange, gel
〃 II	on further addition of 20 cc. alcohol	4.9	faint orange, very viscous
〃 III	residual liquid	7.8	orange yellow, liquid

* : *Cololabis saira* Brevoort.

Table 20. Characteristics of the fractionated oils*.

	Acid value	Sapon. value	Iodine value	Peroxide oxygen**	Oxidized acids %	Mol. weight (RAST)
Fraction I	10.76	211.3	88.92	23.18	14.31	1351
〃 II	7.42	212.4	96.94	23.31	10.56	1093
〃 III	8.47	210.2	100.7	28.82	7.71	978.6

* : Oxidized acids content of the original oil is 9.55%.
** : 0.01 N $Na_2S_2O_3$ in cc., per 1 g. oil.

3. Distribution of the oxidized acids between the soluble fraction and the insoluble fraction of linoxyn.

As it was found that the gelationous film formed on the surface of oil contained an especially large amount of the oxidized acids, it was studied in the

present experiment the problem: in what pattern are distributed the oxidized acids in linoxyn, a typical product of drying-oil?

§ Experimental procedure and result obtained:

Linseed oil was aerated for 10 hours at 120°C. It was then dried in the dispersed light into a film of 2 mm in thickness by making use of a PETRI's Schale. The film was pale yellow to appearance and provided with a rubber-like elasticity. Oxidized acids-content was first determined with a part of this film. After submitting the film to an extraction with ether for 16 hours, oxidized acids determination was again carried out for both the ether soluble- and ether insoluble-fractions. The results are shown in Table 21.

Table 21. Oxidized acids content in linoxyn fractions.

	Yield %	Oxidized acids %
Original linoxyn		34.17
Ether-soluble portion	77	28.41
Ether-insol. portion	23	54.27

As seen in the table, the film under question, "linoxyn," is constituted of an ether soluble fraction amounting to 77% and an ether insoluble one amounting to 23%. As regards the distribution of oxidized acids, a concentration value almost twice as large as that found for the soluble fraction is obtained for the ether insoluble fraction.

§ Discussion:

According to the above three experiments, 1 to 3, it may be said that the oxidized acids are concentrated chiefly in the surface film, namely a gelatinized portion of the oil, especially in an ether insoluble portion of the gel. After all, it is very likely that the oxidized acids bear an intimate correlation to the film formation of a drying-oil.

It is believed nowadays that the physical structure of linoxyn is an apparently homogenous gel which consists of a relatively small amount of highly polymerized fatty acids or glycerides building up a three dimensional network and a relatively large amount of liquid oil.

For the reason of the ever-increasing viscosity of autoxidized oil, some researchers describe that some strongly polar atomic groups such as —CHO and —OH are formed so as to effect a substantial intermolear association. Some other workers attribute this viscosity increase to the formation an iso-colloid consisting of a suspension of polymerized oil in monomeric one[43]. At any rate, it may be considered that such a rise of viscosity is a prelude to the development of a network structure throughout the bulk of the oil.

It has already been noted that the oxidized acids can be fractionated into hydroxy acids, carbonyl compounds, and noncarbonyl compounds, and also that the non-carbonyl fraction may be regarded as polymerized fatty acids from their insolubility in petroleum ether.

Supposing that the oxidized acids are correspondent to the network forming component, phenomena related to drying of oils may be explained in a satisfactory manner. According to the above experiments the oxidized acids are contained far more abundantly in the gel fraction than the liquid phase; and in the case of linoxyn they are present in the ether insoluble fraction by an amount twice as

much as that in the ether soluble fraction. Such distribution of oxidized acids may be a matter of course so long as the supposition is accepted.

Sometimes oxidized acids can be obtained from autoxidized oil in spite of that the viscosity of the oil has not increased to any appreciable extent. In such a case, it may be supposed that the oxidized acids, the building-stone for the network, is too small in amount to be get interlocked.

It is a well known fact that linoxyn decomposes slowly in a long exposure to direct sunshine. In this case the author has found that the oxidized acids content is also decreased in proportion to the progress of the exposure; the result is shown in Table 22.

Table 22. The amount of the oxidized acids contained in a linoxyn exposed to a direct sunshine.

Duration of exposure (days)	Saponification value	Content of oxidized acids (%)
0	286.3	34.17
120	364.1	40.18
170	389.5	36.47
230	399.2	29.60

This fact seems also to support the author's view mentioned above that the oxidized acids constitute the skelton of linoxyn gel structure.

In the above viewpoint, it may be said that FAHRION's oxidized acids and linoxyn acid of linoxyn are by no means different substances.

Remarks about the Effect of Sunlight on the Discoloration

MUNZERT[8] has explained why the discoloration of oil hardly occurs in direct sunlight on the ground of his observing that the oxidized acids become decolorized if kept exposed to the sun. DENSTADT[21], further, from his experience that discolored oils, though decolorized in the sun, become colored again if placed in the dark, considers that the colored substance turns colorless through absorption of energy of short-waved beam but returns to the colored substance, emitting the energy, in the dark. The view that the sunshine and the dark change the color of oil in a reversible way has been proposed also by EIBNER[23].

The present author, on the other hand, has repeatedly confirmed that the oxidized acids, but not oil, once decolorized in direct sunlight, never resume their color even if put back in the dark.

As regards the yellowing of white paint or the rusting of marine products, therefore, the phenomenon seems to be explained more reasonably by considering the relative predominance of decolorization and new formation of the oxidized acids. Namely, while the rate of decolorization is larger than that of formation in the sunlight, the former is smaller than the latter in the dark.

CHAPTER III. IDENTIFICATION OF THE COLORED ENTITY

Section 1. Mother Substance of Colored Material in Discolored Oil

In the preceding section, it has been found that substances responsible for the color of the oxidized acids can be isolated from the latter almost wholly as a fraction of carbonyl nature. This situation naturally suggests the possiblity of that, in the case of discoloration of autoxidized oil too, the oil is already attached by some carbonyl compounds prior to its discoloration and these compounds only acquire a strong color so as to discolor the entire oil when affected by the second factor. And this possibility will turn out a fact according to the experiments to be described below in the present section.

Experiment 14.

§ Experimental procedure and result obtained:

A highly purified oleic acid was put first on a sheet of filter paper and submitted to oxidation at room temperature in dispersed light for 23 days. After the extraction of oleic acid with ether followed by distillating-off the latter, the peroxide oxygen content was estimated to be 50.3 (as number of cc. of 0.01 N $Na_2S_2O_3$ per 1 g of sample oil).

In isolating carbonyl compounds from this practically colorless preparation of oxidized oleic acid, the following two ways were adopted, the isolated individuals being united in the end:

In the first way, a part of the preparation was divided into carbonyl-and noncarbonyl-fractions by a method almost the same as the Girard's employed in the previous section.

In the second way, another part of this same preparation was dissolved in ether and treated with a concentrated aqueous solution of sodium bisulfite. Carbonyl compounds were recovered not only from the precipitate there formed but also from the aqueous mother liquor by decomposing the addition product with sulphuric acid.

The carbonyl compounds obtained in these two ways were dissolved together in ether, and fractionated from the ether solution into neutral-and acidic-carbonyl fractions by means of sodium bicarbonate.

On the other hand, to make sure of that any carbonyl compounds of a novel character are not produced in these processes, two procedures just the same as above were applied respectively to other duplicate samples taken from the same preparation of oleic acid, while this time the acid was previously heated for 14 hours in nitrogen gas until it became completely free of peroxides; a quite similar result was attained to the case without such preheating.

No matter whether the sample oil was accompanied by peroxides or not, all the three fractions obtained, non-carbonyl, neutral carbonyl, and acidic carbonyl, were respectively found to behave quite characteristically as to their manners of coloring:

The noncarbonyl fraction looks yellow or orange-yellow. It contains practically none of peroxides. Though this fraction turns orange-red on the application of alcoholic potash, the color fades away if acidified with mineral acid.

The neutral carbonyl fraction appears orange-colored, and is perhaps accompanied by heptyl- and nonyl-aldehydes, for its smells distinctly of them. This fraction becomes reddish brown when warmed in the presence of alcoholic potash, but decolorized down to yellow if acidified with mineral acid.

The acidic carbonyl fraction, in the last place, is an orange-yellow oily substance whose greater part tends to crystallize gradually at room temperature. It smells like acetic acid. This fraction is turned darkly brown not only by the action of a basic substance or various reagents listed in Tables 4 and 5, but also, to be a notable fact, even by mere slight heating. It is not decolorized any longer, if once colored even when treated with mineral acid.

According to the present experiment, peroxide value for the noncarbonyl fractions isolated with GIRARD–P reagent and sodium bisulfite, from the oxidized oleic acid, whose peroxide value was intitially 50.3, was found to have been practically zero. This result may certainly be traced to the peroxide-decomposing action of the carbonyl-precipitant employed. In a course of reductive decomposition of peroxides, carbonyl compounds are formed in general. In this experiment, therefore, it is not unlikely that the carbonyl compounds-precipitants have combined not only with the carbonyl compounds primarily formed through autoxidation of the raw oil, but also with such secondarily formed by the decomposition of peroxides, which have also been produced in the autoxidation course.

However, since similar results were attained, as mentioned above, independently of whether the sample oleic acid was attended by peroxides or not, it may well be said that the mother substance for the darkly colored material in the oxidized acids is the acidic carbonyl compound which is formed presumably in autoxidation of oil*.

Section 2. Behaviors of Alkali and Acid toward Carbonyl Compounds

In the preceding section the author has figured a view that the colored substance in the oxidized acids, which he considers as a color-developing essential of discolored oil, may have been derived from acidic carbonyl compounds produced in autoxidation of the raw oil.

In the present experiment the mode of discoloration as well as the solubility of nineteen examples of pure carbonyl compounds, which they show on treatment with several reagents familiarly used in separation of the oxidized acids, e.g., alcoholic potash followed by mineral acid, was investigated in parallel with properties of the oxidized acids.

* It has already been predicated that the discoloration of oil may be intimately related to the formation of carbonyl compounds in the course of autoxidation of the oil. But as to what member of them really meets the predication has not been given no comment [ELM, A.C: Ind. Eng. Chem., **24**, 593 (1932); MILAS, N.: Chem.Rev., **10**, 350 (1932); OBATA, et al.,: Bul. Japan. Soc. Sci. Fish., **17**, 13 (1952)].

Experiment 15.

§ Experimental procedure:

For the undermentioned carbonyl compounds the intensity of discoloration they attained on the application of alcoholic potash or gaseous ammonia was examined in the first place. These discolored samples were then acidified with hydrochloric acid and their final colors were examined.

§ Carbonyl compounds used:

As examples of aliphatic saturated aldehydes, acetaldehyde, propionaldehyde, heptylaldehyde, caprylaldehyde, nonylaldehyde, and caprinaldehyde were adopted. For the purpose of purification, they were first distilled in an atmosphere of nitrogen at their respective boiling points mentioned in Table 23; they were then converted into the addition product of sodium bisulfite. The addition product was washed thoroughly with ethyl alcohol and decomposed subsequently with aqueous sodium hydroxide. The aldehyde there liberated was collected in peroxide-free ether, and from this ether solution the ether was gently evaporated-off, free aldehyde being obtained. In melting point of semicarbazone the aldehyde specimens agreed well with their references as shown in Table 23.

Table 23. Boiling point of aliphatic aldehydes and observed melting point of their semicarbazones

Aldehyde	B.P. °C./mm.	M.P. of semicarbazone
Acetaldehyde	21/760	162°C.
Propionaldehyde	49/760	88
Oenanthaldehyde	66—67/41	109
Caprylaldehyde	71—72/20	101
Peralgonaldehyde	80—82/13	100
Caprinaldehyde	93—94/12	102

Table 24. Boiling or melting point of ketonic acids and melting point of their 2.4-dinitrophenylhydrazones (observed).

Ketone	M.P. or B.P. °C./mm.	M.P. of 2.4-DNPH
Diacetyl	b.p. 88/760	314°C.
Pyruvic acid	〃 60–61/12	213
Acetacetic ester	〃 50–60/7	93
9.10-Hydroxyketostearic acid	m.p. 63–64	146.8
9.10-Diketostearic acid	〃 83–84	158

As instances of keto-acid or its derivative, pyrvic acid, acetacetic ester, diacetyl, 9, 10-hydroxyketostearic acid, and 9, 10-diketostearic acid were preferred. The first three members came to hand from the market and purified by fractional distillation in an atmosphere of nitrogen at the boiling point mentioned in Table 24. 9, 10-hydroxyketostearic acid and 9, 10-diketostearic acid were prepared by

the KING's method[44] and the HOLDE-MARCUSON's method[45], respectively. Boiling- or melting-points of thus prepared samples and melting points of their 2, 4-dinitrophenylhydrazones are shown in Table 24.

Of half aldehydes the following six members were adopted as examples:

a) Glyoxylic acid: prepared by electrolytic reduction of oxalic acid[46].

b) Succinic half aldehyde: Acetalyl malonic acid diethyl ester (b.p. 145–160°/15 mm. Hg), which had been prepared from chloroacetaldehyde diethylacetal and monosodium diethyl malonate, was sealed in a long glass tube with water and submitted to hydrolytic decomposition in a tube furnace. Succinic half aldehyde was obtained by fractional distillation at 132–136°/15 mm. Hg[47] of the ether solube portion of this decomposition product.

c) Glutaric half aldehyde: Cyclopentene, which had been synthesized from adipic acid by way of cyclopentanone and cyclopentanol, was ozonized under cooling; then the ozonide was submitted to hydrolysis. Glutaric half aldehyde was obtained from the ether soluble portion of the product by fractional distillation at 150–161°/12 mm. Hg.

d) Adipic half aldehyde: Cyclohexene obtained on the marked was first purified by distillation and then ozonized under cooling. After the ozonide was submitted to hydrolysis, adipic half aldehyde was obtained from the ether soluble portion of the product by fractional distillation at 139–140°/10 mm. Hg.

e, f) Azelaic and sebacic-half aldehyde: these were prepared by oxidative cleavage of 9, 10-dihydroxystearic acid (m.p. 132°C.) and 10, 11-dihydroxyundecanoic acid (m.p. 85–86°C.), respectively, with lead tetracetate.

Melting points and colors of these six half aldehydes, and also the results of ultimate analysis as determined for their 2, 4-dinitropheylhydrazone are shown in Table 25.

Table 25. Ultimate analysis and melting point of half aldehyde 2.4-dinitrophenylhydrazones.

Half aldehyde	Ultimate analysis	C %	H %	N %	M. P.	Color
Glyoxylic acid	calc.	37.77	2.36	22.10	183°C decomp.	yellow
	found	37.80	2.39	21.97		
Succinic-	calc.	42.60	3.54	19.86	201	orange yellow
	found	42.81	3.62	19.83		
Glutaric-	calc.	44.70	4.06	18.94	178	〃
	found	44.92	4.15	18.08		
Adipic-	calc.	46.50	4.52	18.08	142	〃
	found	46.70	4.59	18.15		
Azelaic-	calc.	51.10	5.69	15.90	120—1	〃
	found	50.96	5.63	16.09		
Sebacic-	calc.	52.50	6.01	15.29	95	yellowish orange
	found	52.67	6.27	15.31		

As examples of unsaturated aldehydes, the following were adopted:

a) Crotonaldehyde: prepared by treating acetaldehyde with 15% sodium sulfite[49] at 0°C.

b) Acrolein: prepared by dehydration of glycerine with potassium bisulfate[50].

Melting point of 2, 4-dinitrophenylhydrazone of the acrolein was 165°C., being in good agreement with that of reference. Whereas melting point of the 2, 4-dinitrophenylhyerazone of the crontonaldehyde, a red-colored substance, was 192.5°C. as somewhat higher than that of reference, 190°C., the data of its ultimate analysis showed good agreement with those of theory (as given in parentheses). Namely, C: 47.89% (48.10%), H: 4.24% (4.00%), N: 22.40% (22.41%).

§ Result of experiment.

The acetaldehyde was turned reddish brown or red by the action of alconolic potash or ammonia, respectively. Either of these discoloration products was insoluble in aqueous NaOH, and not decolorized even if added with mineral acid.

The higher homologues of acetaldehyde here tested became yellow- or orange-colored by the action of alcoholic potash, but such discoloration products were decolorized almost perfectly by the addition of mineral acid. These aldehydes were noteworthy in respect of that ammonia was unable to discolor.

The diacetyl turned reddish brown when affected by alcoholic potash and continued to be discolored even if acidified subsequently. The acidified substance was soluble in aqueous sodium hydroxide, alcohol, and ether, but insoluble in petroleum ether. The diacetyl was not discolored by the action of ammonia at room temperature, but turned red when heated with ammonia. The sample treated in this manner was no longer precipitated if acidified, but discolored further to dark brown.

The pyruvic acid was turned brownish-orange by the action of alcoholic potash, but the product became decolorized down to yellow by the addition of mineral acid. When added with ammonia, the pyruvic acid changed pale yellow in color.

The acetacetic ester became colored yellow only by the action of alcoholic potash.

The 9, 10-hydroxyketostearic acid was not discolored at all by the action of alcoholic potash or ammonia.

The 9, 10-diketostearic acid was turned brown by the action of alcoholic potash, but the color was weakend down to pale yellow if acidified subsequently. Application of ammonia to this preparation did not cause discoloration.

The half aldehydes were discolored more or less strongly by the action of alcoholic potash or ammonia, and the larger the number of their carbon atoms, the stronger the discoloration. Discoloration products of the homologs higher than glutaric half aldehye were not decolorized by acidification, while those of the lower members were decolorized. The acidified substances, even after their decolorization, remained insoluble in ether and petroleum ether, but were soluble in alcohol, acetone, and aqueous alkali solutions, in good agreement with the solubilities of the oxidized acids.

The acrolein produced a coloreless polymer by the action of alcoholic potash.

The crotonaldehyde became yellow spontaneously in the air. If affected by alcoholic potash, it changed brown in color; but the color faded slowly back, when acidified, down to the original color, a precipitate being formed. This precipitate was rather very soluble in petroleum ether.

§ Discussion:

As well-known, the oxidized acids are soluble in alcohol, acetone, glacial acetic acids, and alkali solutions, but insoluble in ether, benzene, petroleum ether, and acid solution; they are never decolorized by acidification.

In respect of solubility, therefore, the homologs of half aldehydes higher than glutaric half aldehyde seem to behave quite in concordance with the oxidized acids. The diacetyl and the crotonaldehyde, with the exception of the solubility in ether or petroleum ether, respectively, also closely resemble the oxidized acids in respect of the solubility of alkali-treated substance in acid solution.

Accordingly, it is very probable that the mother of the colored substance in the oxidized acids may be half aldehyde homologs higher than glutaric half aldehyde. The author's assumption that certain compounds belonging to acidic carbonyl ones are the mother substance seems to be favored by this result. Diacetyl, if be existent in autoxidized oil, may contribute to some extent to the coloration of the oxidized acids.

In saponification of autoxidized oil, it is usual that a darkly colored mixture is produced. In this case, with the coloration of the saponified mixture may be concerned not only half aldehydes, but also other carbonyl compounds as well. It is considered that some of the colored substances formed in the usual process of saponification will be of such a nature that they become decolorized if brought in an acidic medium.

Crotonaldehyde, if present in autoxidized oil, may behave as one of sources of the yellowing of purified fatty acids and oils stored in the air.

Section 3. Paper Chromatography of Acidic Carbonyl Compounds

In the preceding sections the author have found two remarkable facts: 1) The color of the so-called oxidized acids originates in their containing carbonyl compounds, if in a relatively small amount, or, speaking more precisely, acidic carbonyl compounds. 2) The half aldehyde homologs higher than glutaric half aldehyde, which are expected of their presence in these carbonyl compounds, become parmanently discolored, when they are affected by the second factor like alcoholic potash, etc. Thus, if once discolored, they are no longer able to be decolorized even in an acidic medium and behave themselves in a variety of solvents quite similarly to the so-called oxidized acids.

From these findings the author has advanced to the proposal that the colored substance in the oxidized acids may be derived, if in part, from the homologs higher than glutaric half aldehyde.

However, such a proposition, of course, will end in a mere assumption if any member of the half aldehydes in question is not proved of its existence in natural autoxidized oil. Now, since half aldehydes are in general far much unstable compared with neutral al'ehydes and readily subject to oxidation, polymerization, and other changes, their presence in natural autoxidized oil, if be the case, is expected to be very minute in amount. Thus, even azelaic half al ehyde, which can be looked upon as the most popular one of half aldehydes, has not as yet been reported of its successful identification in natural autoxidized

oil. The present author, nevertheless, with confidence of that the discoloration of autoxidized oil may surely be due to the discoloration of some concomitant half aldehydes, tried to verify the actural presence of such aldehydes with resort to a paperchromatographic procedure. The experimentals in this connection, where the author studied the behaviors of 2, 4-dinitrophenylhydrazones of various carbonyl compounds, will be given below.

A number of reports of paperchromatography have been issued for neutral carbonyl compounds hitherto, but only a few for acidic carbonyl compounds. CAVALLINI et al[51]., say that they obtained a satisfactory result of paperchromatography for pyruvic acid 2, 4-dinitrophenylhydrazone by using a butanol-ethanol mixture as the developing-solvent. In their photographs, however, the spot looks so broadly spread that their method, be sure as it may be available for fixation of pyruvic acid alone, seems by no means to be so with several materials mutually close in R_f value.

The author, after testing a number of developing-solvents, found that a mixture composed of hexane, glacial acetic acid, and xylene (5:1:3) was quite excellent in respect of its giving compact and discrete spots for 2, 4-dinitrophenylhydrazones of acidic carbonyl compounds.

Although the R_f in this case tends a little to fluctuate as in the case of neutral carbonyl compounds which have been studied by other workers using various solvents, it has been ascertained that identification can successfully be performed with this new solvent, if an authentic specimen of likely hydrazone is run in parallel with the sample to be known.

It should also be remembered that, if this developing-solvent is applied to 2, 4-dinitrophenylhydrazone of neutral carbonyl compounds, the hydrazones are not resolved into any discrete spots, but instead spread over whole surface of the filter paper.

R_f's for 2, 4-dinitrophenylhydrazones of a few acidic carbonyl compounds, observed in using this solvent, are listed in Table 26.

Table 26. R_f of 2,4-dinitrophenylhydrazone of acidic carbonyl compounds and color of the spot developed by N/10 NaOH aq.

2,4-DNPH	R_f	Color
Pyruvic acid	0.41	OY
Glyoxylic acid	0.07	OP
Succinic half aldehyde	0.44	PO
Glutaric half aldehyde	0.55	PO
Adipic half aldehyde	0.60	PO
Azelaic half aldehyde	0.63	PO
Sebacic half aldehyde	0.66	PO
9,10-Hydroxyketostearic acid	0.75	OY
9,10-Dikestotearic acid	0.52	OY

Developing-solvent : hexane, gracial acetic acid, xylene (5 : 1 : 3), Temperature : 25°C.
O : orange, P : purple, Y : yellow.

Section 4. Detection of Half Aldehyde in Autoxidized Oils

In concluding the present series of study, the author intends to verify the actual presence of half aldehydes in autoxidized oils, for he has already arrived at the conviction that the coloration of discolored oil is allowed to become condensed almost entirely to the coloration of half aldehydes which is presumed to be borne in autoxidation of oils.

Experiment 16.

§ Sample oils:

Oleic acid, linolenic acid, and fresh sardine oil were separately applied to large pieces of filter paper and submitted to autoxidation for 30 days in dispersed light at room temperature. They were then extracted from the filter paper with peroxide-free ether. After the ether was evaporated-off under reduced pressure, the residues were heated for 15 hours at 70°C. in an atmosphere of nitrogen so that they might become free from peroxide.

§ Experimental procedure:

Two halves of each autoxidized sample oil prepared above were treated with a concentrated solution of sodium bisulfite and GIRARD-P reagent, respectively. Carbonyl compounds obtained from the two halves were dissolved collectively in ether. The ether solution was then treated with an aqueous solution of sodium bicarbonate, whereby the collected carbonly compounds were fractionated into neutral- and acidic-carbonyl compounds. Both the carbonyl compounds were further converted into their respective 2, 4-dinitrophenylhydrazones.

The 2, 4-dinitrophenyldrazones of the neutral carbonyl compound were submitted further fractionation by the CHANG's[52] procedure. The fractional hydrazones respectively collected from the equivalent bands in 4—6 runs were rechromatographed and recrystallized from ethyl alcohol, separately. The individual hydrazones were identified by measuring their melting points and nitrogen contents in comparision with authentic samples of carbonyl compounds.

The 2, 4-dinitrophenylhydrazones of the acidic carbonyl compounds, on the other hand, were dissolved in a small quantity of chloroform, and the solution was poured on a column of silica gel, which was previously heated for 5 hours at 100°C. Following the chloroform solution, the column was percolated with petroleum ether. Chromato-bands were then made to develope by means of mixtures of ethyl alcohol and petroleum ether, the concentration of ethyl alcohol in which was raised gradually. After the completion of the development, the silica gel column was drawn out from the glass cylinder, the adsorption bands being cut off separately, The corresponding bands from separate runs were united, and the hydrazones were extracted with glacial acetic acid. The extract was concentrated under reduced pressure, and the hydrazones were further fractionated by paper chromatography as described in the preceding section. The R_f values attained were examined in parallel with those for the authentic specimens.

In the case of oleic acid in particular, 2, 4-dinitrophenylhydrazone of azelaic half aldehyde was obtained collectively from the paper-chromatographic spot of R_f equivalent to the spot of a standard of this hydrazone by cutting them out from 65 times repeated runs. The hydrazone obtained was recrystallized, measured of its melting point, and submitted to ultimate analysis.

§ Result obtained:

Carbonyl compounds identified in this experiment are listed in Table 27. Besides those appearing in the table, there were found many individuals of a neutral carbonyl form or of an acidic one, although they unfortunately eluded identification because of their minuteness in amount or lack in authentic samples to be referred to.

Table 27. 2,4-Dinitrophenylhydrazones (2,4-DNPH) of carbonyl compounds isolated from autoxidized oils.

Authentic carbonyl compds. 2,4-DNPH		Carbonyl Compd. found in autoxidized		
Name	M.P.; Color; N%	Oleic acid	Linolenic acid	Sardine oil
Acetaldehyde	163.5~164.5°C.; Y ; N : 25.0%	—	159~162°C.; Y ; N : 24.86%	160~163°C.; Y ; N : 25.18%
Propionaldehyde	155°C.; Y ; N : 23.52%	152~154°C.; Y ; N : 22.79%	153~154°C.; Y ; N : 23.43%	—
Oenanthaldehyde	105°C.; Y ; N : 18.9%	103~105°C.; Y ; N : 18.89%	102~104.5°C.; Y; N : 18 96%	102~104°C.; Y ; N : 18.86%
Peralgonaldehyde	106°C.; Y ; N : 17.4%	104~106°C.; Y ; N : 17.42%	105~106°C.; Y; N : 17.36%	—
Caprinaldehyde	105.5°C.; Y ; N : 16.65%	—	101~104°C.; Y ; N : 16.62%	—
Crotonaldehyde	192.5°C.; R ; N : 22.4%	—	189~192°C.; R ; N : 22.38%	188~192°C.; R ; N : 22.37%
Azelaic half aldehyde		+*	+	+
Sebacic half aldehyde		—	?	+
Diketostearic acid		+	+	+

\+ Means positive existence.

O : orange, R : red, Y : yellow.

*

	M.P.	Mixed m.p.	Color	C %	H %	N %
Authentic specimen	120°C.		OY	51.1	5.69	15.9
Sample	117~120°C.	117~120°C.	OY	51.19	5.54	15.6

§ Discussion:

In additon to the previous attainment that acidic carbonyl compounds in autoxidized oleic acid most strongly potentiate the oil to discolor, it has now been

proved that, at least in most common autoxidized oils examined in this secton, azelaic half aldehyde, and also sebacic half aldehyde in the case of sardine oil, is actually present as a substantial example of such half aldehydes.

It will not be, hence, to much to say that discoloration of oil in general is caused in the main, if not entirely, by discoloration of half aldehydes, for the formation of which the autoxidation of the raw oil is considered to be responsible.

As shown in Table 27, besides a number of neutral carbonyl compounds, diketostearic acid was detected in the present experiment. Some of these compounds become colored fairly strongly under the effect of a basic substance as already mentioned.

Surely, these compounds may participate with half aldehydes in the discoloration that occurs in saponification of oil, or generally speaking, in the presence of the second factor. Nevertheless, as their color vanishes again always in an acidic medium, they cannot be looked upon as the coloring component proper of the oxidized acids.

It is remined on this occasion that purified colorless preparations of fatty acids or of their esters often become yellow-colored while kept stored. As regards the mother substance of this yellow color little has been attacked by the author as yet. However, if it is taken account of the fact that crotonaldehyde is present as shown in Table 27, it is not unlikely that the strong coloring tendency of this aldehyde bears an important correlation even to the yellowing of the said preparations.

SUMMARY

The aim of the present studies is to make clear what it is at all that are truely essential to the occurrence of discoloration of fish oil. It may be expected that through resolution of this problem a clue can be acquired to a proper means of protecting a variety of marine products from being rasted. The results obtained are summarized as follows:

1) For the occurrence of discoloration of fish oil it is always necessary that the oil submits first to autoxidation to some extent. But mere oxidation hardly results in the discoloration; and if a certain substance such as volatile basic nitrogen compound comes in contact with this oxidized oil, first then starts the discoloration. The author defines the preparative conditions established through the autoxidation as the first factor and the substances inducing the autoxidized oil to color as the second factor in the discoloration mechanism.

2) A considerable number of substances belonging to inorganic and organic fields, whether basic, acidic, or neutral in common sense, are recognized to be able to behave as the second factor. So long as dehydrated or refrigerated fish is concerned, the entity that plays the role of the second factor is not its bacterial contamination but rather the volatile basic nitrogen compounds present in the raw fish; the most serious subject to be called to account here is the degree of freshness of the raw fish.

3) The essential substance coloring the discolored fish oil, although may

be derived mainly from rather highly unsaturated fatty acids in natural discoloration, should be traced as well to fatty acids of lower unsaturation and saturated *n*-fatty acids containing carbon atoms more than twelve in discoloration *in vitro*. Glycerine, though it develops only a far lighter color as compared with these fatty acids, has some relation to the discoloration. Unsaponifiable matters and saturated fatty acids having carbon atoms below ten bear no relation to the occurrence of discoloration.

4) FAHRION's "oxidized acids" are an essential colored fraction of discolored oil.

5) Chemical characteristics of the oxidized acids from various oils examined following LEWKOWITSCH point to the existence of both unsaturated and lactone (perhaps γ or δ) linkages. A marked difference is found in nitrogen content between different samples of the oxidized acids not only when they are different in the kind of raw material but also when the same in kind but variant in the history before isolation; this is received as one of criterions for the catalytic share of the second factor like ammonia.

6) The correlation found between the quantities of the ammonia added as a second factor to autoxidized oil and the optical densities atained deviates distinctly from a straight line. This indicates that the partaking of the ammonia in the discoloration is not as a partner with which the oil forms some colored compound but rather as a catalyst merely promoting the color-development of certain materials already present in the oil.

7) The dark color of the oxidized acids is attributable to their containing carbonyl compounds in a small proportion, while the main portion of the oxidized acids is composed of colorless substances.

8) Of these carbonyl compounds, acidic carbonyl compounds can be selected out as a more refined source of the dark color.

9) Among the carbonyl compounds investigated, only half aldehyde homologs higher than glutaric half aldehyde are possible to be the mother substance of darkly colored material in the oxidized acids.

10) Paperchromatographic behaviors of 2, 4-dinitrophenylhydrazones of a few acidic carbonyl compounds were investigated. As a developing-solvent quite excellent in allowing the mixture of these hydrazones to be resolved into discrete and compact spots, a mixture composed of hexane, glacial acetic acid, and xylene in the ratio 5:1:3 was offered.

11) By pursuing the carbonyl compounds present in autoxidized oleic acid, linolenic acid, and sardine oil, the presence of the following carbonyl compounds was verified in the respective samples:

The oleic acid: propion-, oenanth-, peralgon-aldehyde, azelaic half aldehyde, and diketostearic acid; the linolenic acid: acet-, propion-, oenanth-, peralgon-, caprin-, croton-aldehyde, azelaic half aldehyde, diketostearic acid; the sardine oil: acet-, oenanth-, croton-aldehyde, azelaic-, sebacic-halfaldehyde, and diketostearic acid.

12) In the discoloration of autoxidized oil accomplished first by the help of the second factor, like the rusting of certain fish products or the coloring of

a usual saponification mixture, various sorts of carbonyl compounds formed in the course of autoxidation, such as aldehydes of saturated fatty acids, diketostearic acid, half aldehydes, etc., are equally regarded to be the anticedents of the coloring material. But, if it is confined to the color of the oxidized acids fraction, the coloring material is derived almost entirely from half aldehyde homologs higher than glutaric half aldehyde.

13) At variance with the case of yellowed white paint or rusted fish products, the oxidized acids once decolorized in direct sunlight never resume their color even if kept again in the dark. The known recoloring in the dark of sun-decolorized white paint or fish products is given an improved account by assuming not recovery but rather accumulation of colored oxildized acids formed anew.

References

1) BEDFORD, R. H.: Biol. Board Can., No. **27**, 11 (1936).
2) DAVIS, W. L., and GILL, E.: J. Soc. Chem. Ind., **55**, 141 T (1936).
3) OTTO, B.. Z. Unters. Lebensm., **73**, 74 (1937).
4) OBATA, Y., et al.: Bul. Japan. Soc. Sci. Fish., **15**, 412 (1949).
5) TSURUGA, H., et al.: Bul. Naikai Regional Fish. Res. Lab., (Japan) No. **2**, 34, 40 (1952).
6) LEA, C. H.: J. Soc. Chem. Ind., **53**, 182 T (1934).
7) NAUROY, A.: Paintures pigments vernis, **6**, 997 (1926).
8) MUNZERT, H.: Farben-Ztg., **33**, 2849 (1928).
9) COLOMB, P.: Ind. Vernice (Milan), **4**, 64 (1950); Chem. Abst., **44**, 10346 (1950).
10) TOMIYAMA, S., et al.: J. Japan Oil Chem. Soc., **1**, 17 (1952).
11) BOLAM, T. R., and SIM, W. S.: J. Soc. Chem. Ind., **60**, 50 (1941).
12) SALMON, G.: Ind. Vernice (Milan), **4**, 142 (1950).
13) MUKAI, K.: Bul. Japan. Soc. Sci. Fish., **20**, 242, 245 (1954); **21**, 187 (1955).
14) VICKERY.: Rept. of the Food Invest. Board for the year **1927-8-9**.
15) LEA, C. H.: J. Soc. Chem. Ind., **50**, 343 T (1931).
16) EIBNER, A.: Paint varnish production mgr., **14**, 12, 14, 30 (1936).
17) SCHEIBER, J.: Farbe u. Lack **1931**, 511-12.
18) ELM, A. C.: Ind. Eng. Chem., **23**, 881 (1931).
19) UENO, S. and KOMORI, S.: J. Soc. Chem. Ind., Japan, **37**, 1444 (1934).
20) SMITH, Jr. F. G.: J. Am. Oil Chem. Soc., **25**, 328 (1948).
21) DENSTADT, O. F. and BROCKLESBY, H. N.: J. Biol. Board Can., No. **2**, 13 (1936).
22) TSUJIMOTO, M.: "Kaisan-dobutsu Yu" or "Oils of Marine Animals" (1918).
23) EIBNER, A.: Paint varnish production mgr.. **13**, 7, 10, 12 (1935); **14**, 12, 14, 30 (1936).
24) KISCLEV. V. S., and RASSUDOVA, N. S.: Chem. Abst., **34**, 5679 (1940).
25) RASSDOVA, N. S., and TOLGSKII, Yu. N.: Chem. Abst., **36**, 1505 (1942).
26) SCOFIELD, F.: Chem. Abst., **35**, 6473 (1941).
27) TAFFEL, A. and REVIS, C.: J. Soc. Chem. Ind., **50**, 87 T (1931).
28) FAHRION,: Z. angew., Chem., **4**, 540 (1891); **11**, 781 (1898); GRUN, A.: Analyse der Fette u Wackse, Bd. **1**, 248 (1925) Berlin.
29) MORRELL, R. S., and PHILLIPS, E. O.: Fette u. Seifen, **46**, 541 (1939).
30) TSUJIMOTO, M.: J. Soc. Chem. Ind., Japan, **15**, 156 (1912).
31) HILDITCH, T. P.: J. Soc. Chem. Ind., **47**, 105 (1928); **49**, 138 (1930).
32) BEILSTEINS HANDBUCH DER ORGANISCHEN CHEMIE Bd. II. (1920).
33) RALSTON, A. W.: "Fatty Acids and Their Derivatives" p. 387 (1948).

34) Morrell, R. S., and Marks, S.: J. Soc. Chem. Ind., **50**, 27 T (1931).
35) Elm, A. C., and Standen, G. W.: Ind. Eng. Chem., **24**, 1044 (1932).
36) Elm, A. C.: Ind. Eng. Chem., **24**, 593 (1932).
37) Pallauf, F.: Seiden-Oele-Fette-Wachse, **75**, 51 (1949); Chem. Abst., **44**, 1267 (1950).
38) Lewkowitsch, J.: "Chemical Technology and Analysis of Oils, Fats, and Waxes", Vol. III., p. 175 (1915) London.
39) Oda, R., and Wada, M.: J. Soc. Chem. Ind., Japan, **37**, 644 (1934).
40) Girard, and Sandulesco: Helv. Chim. Acta, **19**, 1095 (1036).
41) Graf: Chemisches Zent., II, 3337, 3338 (1936).
42) Skellon, J. H.: J. Soc. Chem. Ind., **50**, 382 T (1931).
43) Stewart, J. R.: "The National Paint Dictionary" 3rd. Ed., p. 354 (1948).
44) King, G.: J. Chem. Soc., 1788 (1936).
45) Holde, D., und Marcuson, J.: Ber., **36**, 2657 (1903).
46) Cohen, J. B.: "Practical Organic Chemistry", p. 127 (1930) London.
47) Wohl, A.: Ber., **31**, 1791 (1898).
48) Harries, C. D.: "Untersuchungen Über das Ozon und seine Einwirkung auf organische Verbindungen", s. 401, 416 (1916).
49) Yamaguchi, S.: "Practical Organic Chemistry", (in Japanese), p. 732.
50) Asahina and Shibata: "Organic Systheses" (in Japanese), Vol. I, p. 16.
51) Cavallini, D., et al.: Nature, **163**, 568 (1949); **164**, 792 (1949).
52) Chang, S. S., and Kummerow, F. A.: J. Am. Oil Chem. Soc., **30**, 251 (1953).

Journal of the Tokyo University of Fisheries, Vol. 43, No. 2, 1957

STUDIES ON THE KAMABOKO MADE BY THE ENRICHING OF VITAMINS

Isami Osakabe, Toshiyuki Hirano and Ikunosuke Okada

(Received Feb. 11, 1957)

Introduction

With the purpose of producing higher nutritive value of Kamaboko*, the authors have tried to make the Kamaboko enriched with vitamin A or C, and studied on the change of these vitamins in the manufactured products, and also in each layers of the surface, center and bottom of the manufactured products during the manufacturing process and storage.

Moreover an organoleptic test was made whether these vitamins would produce any effect upon the appearance, odour and other properties of products.

Experimental

In these studies, the experiments were divided into two sections.

The first experiment (Section 1) was performed on the wholeness of Kamaboko, the second (Section 2), performed in three layers, namely the surface, center and bottom layer of Kamaboko.

I. Change of vitamins on the wholeness of Kamaboko.

Kinds of fish used as the raw material were Mezirozame (*Carcharlinus gangeticus*), Yoshikirizame (*Prionace glauca*), and Hamo (*Muracnesox cinereus*).

Surimi** was prepared as the usual method. Fish flesh and other materials employed for the preparation of Surime were mixed as the following proportion (Table 1).

Table 1. Proportional amounts of the raw materials used in Surimi.

Raw materials	Water	Fish flesh	Starch	Na-glutaminate	Saccharin	Glucose	Salt
Proportional amount	16.2 *l*	86 kg	9.4 kg	560 g	30 g	1 kg	2 kg

Vitamin A (the concentrated fish liver oil containing vitamin A, which was emulsified with the surface active agent, Atlox 1045-A) was added to surimi so that 524,700 I.U. of vitamin A per 100 grams of Surimi was in existence. And thus Surimi was put to form semi-cylindrical piles on the wooden plates, cooked by boiling with steam, and then could be made into the Kamaboko enriched with vitamin A.

* Kamaboko—A sort of semi-cylinder-shaped pudding quite popular in Japan, which is made by cooking the fish flesh previously ground with common salt.

** Surimi—A sort of fish-paste prepared by grinding the fish flesh with common salt; it can be made into Kamaboko by cooking after being set into a semi-cylindrical block.

Vitamin C (in general use for enrichment purpose; purity 95% as reduced vitamin C) was added to Surimi so as to be in concentration of 46.6 mg. per cent. Then the enriched Kamaboko with vitamin C was prepared as usual method.

Moisture and crude fat in these samples are shown in Table 2.

Table 2. Amounts of moisture, dry matter and crude fat in Surimi and Kamaboko when vitamin A or C was added.

	Moisture (%)	Dry matter (%)	Crude fat (%)
Surimi enriched with V. C.	76.88	23.12	0.324
Kamaboko enriched with V. C.	76.47	23.53	0.321
Kamaboko enriched with V. C.	76.90	23.10	0.518

To study on the change of vitamins in Kamaboko during the manufacturing process and storage, the contents of vitamin A and C were determined after finishing of Surimi stirred and grinded together with vitamins and of Kamaboko produced. Thereafter the determination was continued every day during the storage of Kamaboko.

The storage temperature of Kamaboko was kept at 25°C., and the storage-period was for four days.

Vitamin A contained in the sample was estimated by the Carr-Price method by the Lovibond Tintometer, after extracting the crude fat with ether from the sample dehydrated with anhydrous Glauber's salt. And vitamin C was estimated by the Indophenol method, after the sample was homogenized with 2% meta-posphoric acid.

The change of the amounts of vitamins during manufacturing process and storage are shown in Table 3.

Table 3. Change of the amounts of vitamin A and C in the manufactured products (Storage at 25°C).

Days	Conc. of reduced V. C.	Residue	Conc. of V. A.	Residue
Surimi (calculated value)	46.6 mg%	100 %	524,700 I.U.	100 %
Surimi (4 hrs. after stirring and grinding)	46.5	99.8	493,000	94.0
Kamaboko (4 hrs. after produced)	38.9	83.7	411,900	78.5
1	38.2	82.1	326,900	62.3
2	35.6	76.6	414,800	79.1
3	33.8	72.7	347,900	66.3
4	29.9	64.3	393,900	75.1

After finishing of the stirring and grinding of Surimi together with vitamins, the amounts of vitamins were not very decreased, but in the process of Kamaboko manufacturing, vitamins added were rapidly deminishing to some extent. In storage they were gradually deminishing.

The results observed by the organoleptic test of the Kamaboko enriched with vitamins are given in Table 4.

Table 4. Comparison of odour, color and taste by the organoleptic test of the Kamaboko enriched with vitamin A or C. And pH value measured by the Beckman glass electrode pH meter. N: Normal, G: Good, B: Bad. (Storage at 25°C)

Kinds of Kamaboko	Not enriched				Enriched with V. A.				Enriched with V. C.			
Days	0	1	2	3	0	1	2	3	0	1	2	3
Odour	N	N	N	N(?)	B	B	B	B	N	N	N	N(?)
Taste	N	N	N	N	B	B	B	B	N(G)	N(G)	N(G)	N
Color	N	N	N	N(?)	B	B	B	B	N	G	G	N(?)
pH	7.05	6.97	6.90	6.80	7.05	6.95	6.90	6.80	7.05	6.96	6.91	6.78

Concerning the appearance of the Kamaboko enriched with vitamin A (contained in fish liver oil), an undesirable results were obtained, but the vitamin A addition had not so many relations with the appearance as vitamin A.

II. Change of vitamins in each layers of the surface, center and bottom of the Kamaboko enriched with these.

In this case, Kinki (*Sebastalobus macrochir*) and Aozame (*Isurus glaucus*) were used as raw materials. And then Surimi was prepared by the mixing proportion of these fish flesh and other materials as shown in Table 1.

In the case of the vitamin A enrichment, the concentrated fish liver oil was added to Surimi by itself so as to be in concentration of 15,800 I.U. per gram and then the vitamin A enriched Kamaboko was prepared as usual method. On the other hand, vitamin C was added to Surimi so as to be in concentration of 39.24 mg. per cent and the Kamaboko enriched with vitamin C was prepared as usual method.

Crude fat in the sample are shown in Table 5.

Table 5. Amounts of crude fat in Surimi and Kamaboko when vitamin A or C was added or not.

Kinds of Surimi	Crude fat (%)	Kinds of Kamaboko	Crude fat (%)
Not added	0.578	Not enriched	0.582
Added with V. A.	5.527	Enriched with V. A.	6.467
Added with V. C.	0.567	Enriched with V. C.	0.541

The experimental method after this, was the same as previously mentioned. But in this experiment, the contents of vitamin A in the sample was determined by the absorption spectra method using the Beckman's DU quartz spectrophotometer after treating the sample with the same way as already mentioned.

The change of the amounts of vitamins extending to three layers during the manufacturing process and storage are shown in Tables 6 and 7, or Figs. 1 and 2.

In this manufacturing process of the Kamaboko enriched with vitamin A and C, the same deminishing tendency as the previous experiment was observed.

Moreover extending to three layers there was higher diminishment of both vitamin A and C in the surface layer than the others.

In this organoleptic test shown in Table 8, the vitamin A addition by fish liver oil had ill influence on the appearance, taste and other properties, but the influence by the vitamin C addition was not almost given ill.

Table 6. Change of the amounts of vitamin A in the manufactured products (Storage at 25°C).

Days	Conc. of V. A (I. U.)			
	Surface layer	Center layer	Bottom layer	
Surimi (calculated value)				15,800
" (4 hrs. after stirring and grinding)				15,500
Kamaboko (5 hrs. after produced)	14,200	14,600	14,900	
1	12,000	13,400	14,800	
2	11,200	12,400	12,400	
3	10,400	11,300	13,200	
4	10,100	11,400	10,400	

Table 7. Change of the amounts of vitamin C in the manufactured products (Storage at 25°C). R.V.C.: Reduced vitamin C.

Days	Surface layer		Center layer		Bottom layer	
	Conc. of R.V.C.	Residue	Conc. of R.V.C.	Residue	Conc. of R.V.C.	Residue
0	39.24mg%	100 %	39.24mg%	100 %	39.24mg%	100 %
1	20.64	52.59	26.95	68.67	24.67	62.87
2	16.81	42.84	24.99	63.68	24.22	61.72
3	15.55	39.65	22.48	57.28	20.15	51.35
4	14.58	37.16	21.32	54.33	19.75	50.33

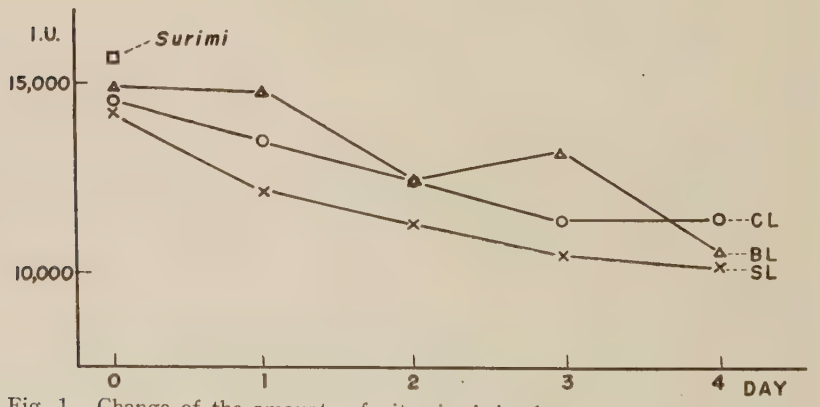


Fig. 1. Change of the amounts of vitamin A in the manufactured products. SL: Surface layer, CL: Center layer, BL: Bottom layer.

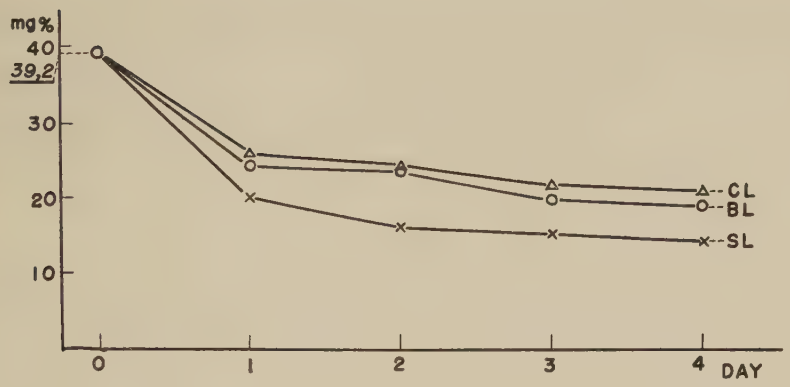


Fig. 2. Change of the amounts of vitamin C in the manufactured products. SL: Surface layer, CL: Center layer, BL: Bottom layer.

Table 8. Comparison of odour, color and taste by the organoleptic test of the Kamaboko enriched with vitamin A or C. And pH value measured by the Beckman glass electrode pH meter. N: Normal, G: Good, B: Bad. (Storage at 25°C)

Kinds of Kamaboko		Not enriched					Enriched with V. A.					Enriched with V. C.				
Days		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Odour		N	N	N	B	B	B	B	B	B	B	N	N	N	B	B
Taste		N	N	N	/	/	B	B	B	/	/	N	N	N	/	/
Color		N	N	N	B	B	B	B	B	B	B	N(?)	N(?)	N(?)	B	B
Neto***		−	−	+	++	+++	−	−	+	++	+++	−	−	+	++	+++
Mold		−	−	−	+	+	−	−	−	+	++	−	−	−	+	+
pH	Surface layer	/	6.45	7.20	6.80	7.10	/	6.40	6.40	6.72	6.85	/	6.40	6.55	7.00	6.85
	Center layer	/	6.40	6.60	6.40	6.55	/	6.40	6.40	6.30	6.18	/	6.55	6.52	6.52	6.40
	Bottom layer	/	6.35	6.35	6.35	6.35	/	6.30	6.35	6.40	6.30	/	6.35	6.40	6.60	6.60

*** Note—Sticky, jelly-like substance appearing on the surface of Kamaboko-block somewhat deteriorated in moisture.

Discussion

As far as this experiment, it was easily observed that vitamin C added nto Kamaboko was destroyed to some extent during the manufacturing process of products.

It was assumed that the destruction of vitamin C was dependent on the function promoting the oxidation of vitamin C by heating Surimi with steam in cooking. And moreover extending to three layers there was higher destruction of vitamin C in the surface layer than the others. It was considered that the destruction of vitamin C was caused by the moisture, light, air, bacteria and mold etc.

In the case of the vitamin A enrichment, the same tendency was found as vitamin C, but it was somewhat stable than vitamin C.

In the organoleptic test, the Kamaboko added with vitamin A was undesirable

in some respects. It was greatly due to the characteristic odour of the fish liver oil containing vitamin A. If the synthetic vitamin A is used, it seems that a desirable effect will be obtained.

On the whole, the enriched Kamaboko with vitamin A or C could be put to use within a few days, but, as regards raw materials, methods of the addition of vitamins, amounts of vitamins added and their storage condition these studies should be carried.

Summary

A trial of producing higher nutritive Kamaboko with vitamin A and C was made, and the change of vitamins in the enriched Kamaboko during the manufacturing process and storage were studied.

(1) The change of the amounts of vitamins used for enrichment was hardly suffered during stirring and grinding of Surimi added with vitamins, but somewhat suffered during the manufacturing process of Kamaboko-products.

(2) Vitamin C added into Kamaboko was easily changeable as compared with vitamin A during the manufacturing process and storage.

(3) The change extending to three layers of the enriched Kamaboko with vitamin A or C was severely suffered in the surface layer of their parts.

(4) In the organoleptic test, the Kamaboko added with vitamin A by fish liver oil was undesirable, if used abundantly, in some respects. But concerning the appearance, odour and other properties Kamaboko added with vitamin C indicated the same inclination of changing as common products.

This study was supported in part by a Grant in Aid for Ministry of Education to which the offers their hearty thanks.

Journal of Tokyo University of Fisheries, Vol. 43, No. 2, 1957

POLAROGRAPHIC STUDIES ON FISH MUSCLE MEATS

Kiyoshi Fukushima, Isami Osakabe, Takeaki Kikuchi
and Ikunosuke Okada

(Received Feb. 11, 1957)

Introduction

We have been studying SH-groups in marine products by polarographic method. SH-groups in various seaweeds, carp or whale meats and viceras were estimated in the preliminary reports[1,2]. Consequently, the authors found that there were much more free SH-groups in the fish viceras than in the muscle meats. It is well known that disulfide or sulfhydryl groups are liberated when the protein molecule is denatured, and the polarographic examination of protein solutions during the denaturation process show, in most cases, an increase in the catalytic protein wave[3]. From the point of view that the denaturation of putrefied muscle proteins would have brought on the changes of free SH-groups, in fish muscle meats were investigated and shown as a reducing power for ferricyanide reagent[4]. There are many analytical methods for the estimation of SH-groups, for the instances, ferricyanide colorimetric (Anson Mirsky's method[5]), polarographic[6]), and amperometric titration[7]) methods. SH-groups and ascorbic acid in the various fresh fruits were determined simultaneously by polarographic method (P. Zuman[8]). In this experiments, SH-groups in fish muscle meats were also determined polarographically. This paper describes the changes of SH-groups in fish muscle meats on the lowering process of their freshness.

Experimental

I. Preparation of sample for the polarographic measurements.

About 10~20g. of ground meats were incubated at 35°C. or allowed to stand at room temperature,

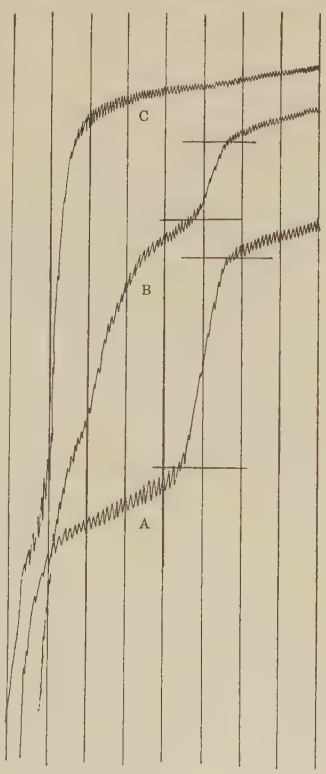


Fig. 1. Anodic waves of sulfhydryl groups.
A, Polarogram of standard glutathione in the supporting electrolyte, McIlvaine buffer. (pH=7.0) and Metaphosphoric acid soln. (5%) (3:1). Concentration, 1.95×10^{-4} M. GSH, Oxidation Potential, −0.24 Volt. vs. S.C.E., Height of Wave, 52.5 mm; B, Polarogram of SH-groups in mackerel ordinary meat extract. Oxidation Potential, −0.281 Volt. vs. S.C.E., Height of Wave, 20.0 mm, C, Above the same supporting electrolyte solution. Galvanometric sensitivity, 1/5=1/5, Anode Potential, Volt. vs. S.C.E.

and SH-groups were determined periodically by polarographic method at the interval of 2 hrs.

The methods for polarographic measurement were as follows, the weighed sample meats were homogenized with 10 ml. of metaphosphoric acid soln. (5% soln.), and 10 ml. of McIlvaine buffer (pH=7.0) was added to the above homogenate, then the mixed homogenate solution was centrifuged and filtered.

The filtrate was used as the sample electrolyte soln. for the polarographic measurement. The pH of electrolyte soln. was usually to be kept on the range of 5.4~5.8. Polarograms of sample and of SH-standard solution (1.95×10^{-4} M. GSH) were shown in Fig. 1. The oxidation potential of the anodic wave of SH-groups in fish muscle extracts are resembled to that of standard GSH solution.

II. Preliminary experiments on mackerell fish muscle meats.

SH groups in mackerell fish muscle meat (ordinary meat) were determined on the lowering process of the freshness, and the results were shown in Table 1. SH contents in meats were all indicated as the mg. percent of GSH. As shown in Table 1, it seemed that the heights of the oxidation waves or SH-contents increased rapidly in proportion to the lowering of the freshness.

Table 1. Mackerel Fish Ordinary Meat.
The change of SH-groups in time. (at the incubation of 35°C.)

Hours	0	2	4	6	8	10	**1.95×10^{-4}M Standard GSH
Wave Height S=1/5	**20.0	25.5	24.5	35.0	38.5	57.0	52.5mm.
Oxid. Potential V	−0.281	−0.292	−0.275	−0.280	−0.272	−0.295	−0.274
SH-Contents* mg%	6.9	8.7	8.4	12.0	13.0	19.5	

Note: * SH-contents are shown as the mg% of glutathione.
** The both polarograms are shown in Fig. 1.
Sample soln: A 20 g. of meat, 10 ml. of metaphosphoric acid soln. (5 %) and 10 ml. of McIlvaine buffer. (pH=7.0).

III. Experiments on bonito fish meats.

The bonito fish meats were allowed to stand at 35°C. temperature, and the changes of SH-groups in ordinary or bloody meats were observed polarographically. Volatile basic nitrogen, and pH value of meats were measured simultaneously. The results were shown in Tables 2 and 3, or Figs 2 and 3. Consequently, the graduary increase of SH contents in ordinary meat run nearly parallel with that of volatile basic nitrogen. On the contrary, SH-contents in the bloody meats decreased on the lowering process of the freshness.

IV. Experiments on carp fish meats.

Carp fish meats were allowed to stand at room temperature and the change of SH-groups in ordinary or bloody meats were investigated polarographically. The results were shown in Table 4. In this experiments, the authors also found

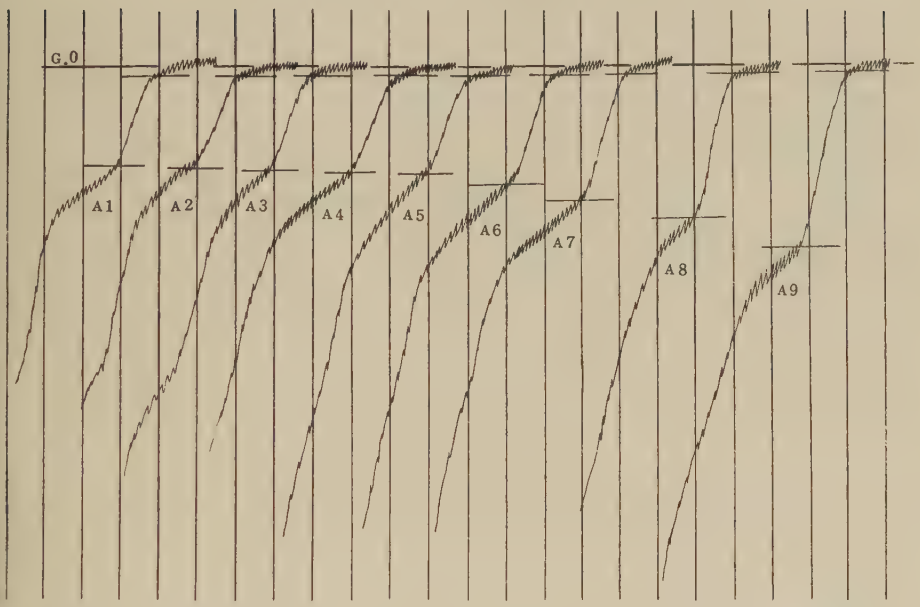


Fig. 2. Bonito fish ordinary meat. The change of polarogram on the lowering process of the freshness. The heights of each waves and the elapsed time are shown in Table 2.

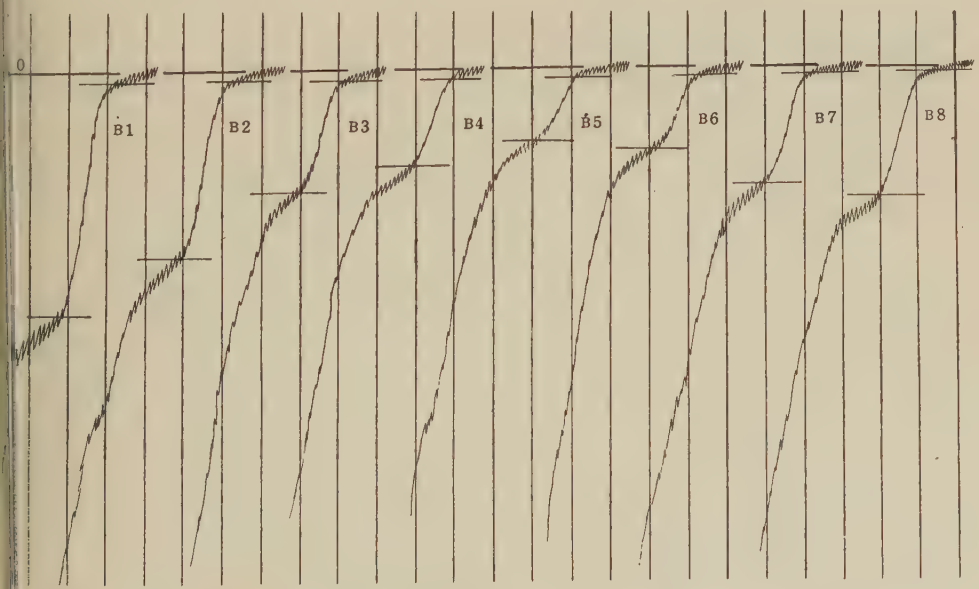


Fig. 3. Bonito fish bloody meat. The change of polarogram on the lowering process of the freshness. The heights of each waves and the elapsed time are shown in Table 3.

Table 2. Bonito Fish Ordinary Meat.
The change of SH-groups, pH and volatile base in time. (at the incubation of 35°C.)

Polarogram	A. 1	A. 2	A. 3	A. 4	A. 5	A. 6		A. 7	A. 8	A. 9	
Hours	0	2	4	6	8	10	12	13	14	15	18
Wave Height mm S=1/5	23.0	22.5	22.0	22.0	24.0	27.0	—	33.5	35.5	40.0	42.0
pH of Elact. Soln.	5.80	5.65	5.35	5.40	5.40	5.60	—	5.40	5.35	5.46	5.58
SH-Contents mg%	10.4	10.4	10.0	10.0	11.0	12.4	—	15.3	16.4	18.4	19.2
Oxid. Potential V.	−0.246	−0.249	−0.253	−0.253	−0.258	−0.259	—	−0.257	−0.279	−0.273	−0.257
pH of Meat	5.90	5.95	6.10	6.10	6.00	6.20	6.25	6.30	6.60	6.70	6.70
Volatile Base mg%	7.70	8.35	8.41	11.05	11.52	12.43	13.65	14.24	14.61	19.05	22.02

Note: SH-Contents are shown as the mg% of glutathion.

Table 3. Bonito Fish Bloody Meat.
The change of SH-groups, pH and volatile base in time. (at the incubation of 35°C)

Polorogram	B. 1	B. 2	B. 3	B. 4	B. 5	B. 6		B. 7				B. 8
Hours	0	2	4	6	8	10	12	13	14	15	16	18.5
Wave height mm. S=1/5	62.0	39.0	31.5	24.0	17.5	22.0	—	27.0	—	—	—	30.0
pH of Elect. Soln.	5.80	5.65	5.70	5.50	5.60	5.45	—	5.70	—	—	—	5.35
SH-Contents mg%	28.4	18.0	14.4	10.8	8.0	10.0	—	12.4	—	—	—	13.6
Oxid. Potential V	−0.284	−0.248	−0.246	−0.238	−0.248	−0.264	—	−0.248	—	—	—	−0.246
pH of Meat	6.10	6.00	5.95	6.35	6.20	6.35	6.60	—	—	6.70	6.90	6.95
Volatile Base mg%	6.91	7.11	9.92	13.88	16.57	16.63	21.04	—	34.47	—	36.27	44.64

Note: SH-Contents are shown as the mg% of glutathion. Sample soln. in Tables 2 and 3. A 10 g. of meat, 10 ml. of metaphoshoric acid soln. (5%) and 10 ml. McIlvaine buffer (pH=7.0). Polarogram are shown in Figs. 2 and 3.

Table 4. Carp Fish Ordinary and Bloody Meats.
The change of SH-groups in time (at the room temp. 8~12°C).

	Days	0	1	2	3	4	5	1.6×10^{-4}M Standard GSH
Ordinary meat	Wave height mm. S=1/2	9.5	12.0	12.0	16.5	23.0	23.0	55.5
	SH-Contents mg%	4.5	6.0	6.0	8.0	11.0	11.0	
	Oxid. Potential V.	−0.250	−0.280	−0.276	−0.274	−0.295	−0.246	−0.280
	Volatile Base mg%	8.0	12.5	17.4	18.4	35.2	57.3	
Bloody meat	Wave height mm	25.0	21.0	20.0	21.0	16.0	9.0	
	SH-Contents mg%	12.7	10.3	9.8	10.3	7.3	3.5	
	Oxid. V.	−0.296	−0.284	−0.290	−0.275	−0.280	−0.264	

Note: SH-Contents are shown as the mg% of gluthion. Sample soln. A 5 g. of meat, 5 ml. of metaphosphoric acid soln. (5%), and 20 ml. of McIlvaine buffer (pH=7.0).

that the changes of SH-groups in carp fish ordinary and bloody meats had been similar to that of bonito on the lowering process of the freshness.

V. Experiments on bonito fish liver.

It is well known that the metabolism of bloody meats is similar to that of liver, and so the authors have made the investigation on SH-groups in bonito fish liver. The results were shown in Table 5 and Fig. 4. The ground liver was allowed to stand at 35°C. temperature for the time of 20 hrs. SH-groups in the liver were determined periodically by polarographic method. As the results, the rapid decrease of them were fairly observed. This changes were assumed to be the same as that of bonito bloody meat.

Table 5. Bonito Fish Liver.
The change of SH-groups in time (at the incubation of 35°C).

Polarogram	C. 1	C. 2	C. 3	C. 4	C. 5	C. 6	C. 7	C. 8	5.2×10^{-4}M
Hours	0	2	4	6	8	10	14	20	Stadard GSH
Wave height. S=1/10	48.5	48.0	24.5	15.5	11.5	11.0	10.0	0	50.5
Oxid. Potential	−0.358	−0.366	−0.348	−0.352	−0.342	−0.356	−0.351	—	−0.366
Anode. Potential	+0.422	0.424	0.422	0.423	0.408	0.414	0.414	—	0.409
SH-Contents mg%	152.0	150.0	76.8	48.5	36.0	34.5	31.4	0	

Note: SH-Contents are shown as the mg% of glutathion. A 20 ml. of McIlvaine buffer (pH=7.0) was added to 2 g. of liver, and the filtrate of homgenate soln. was immediately used as the sample electrolyte. Polarograms are shown in Fig. 4.

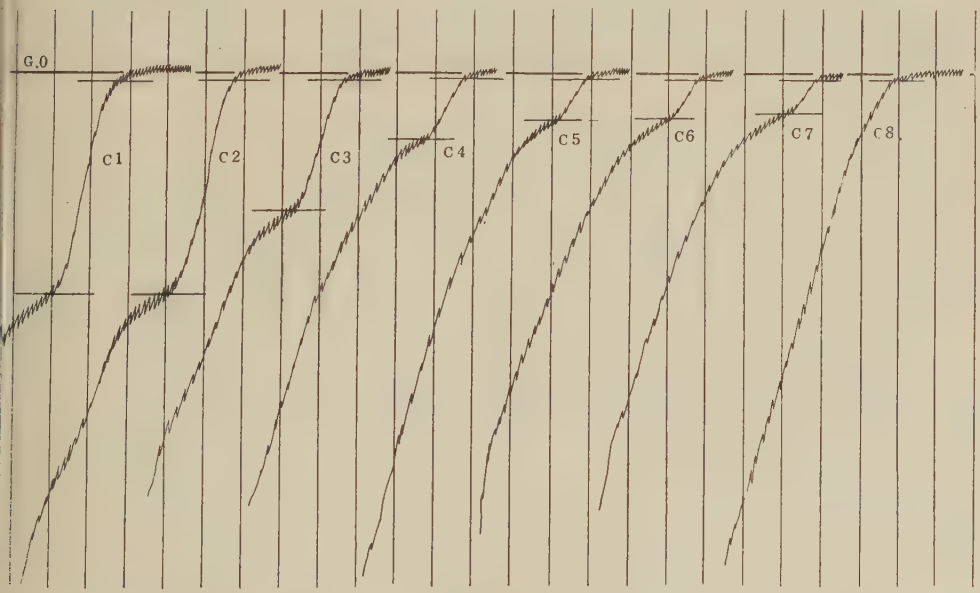


Fig. 4. Bonito fish liver. The change of polarogram on the lowering process of the freshness. The heights of each waves and elapsed time are shown in Table 5.

Résumé

SH-groups contents in fish meats were investigated polarographically on the lowering process of the freshness, and the authors have observed on the camparison between the changes of SH-contents in ordinary meats and bloody meats. The results were as follows.

(1) SH groups in mackerel, bonito and carp fish ordinary meats increases slowly in proportion to the lowering of the freshness.

(2) The increasing rate of SH groups seems to be much more (rapid) in mackerel than in bonito or carp fish ordinary meats, as far as we examined.

(3) SH-groups are contained much more in the fresh bloody meat or liver than in the fresh ordinary meat, and that of bonito and carp fish bloody meats decrease rapidly up to the beginning time of putrefaction.

(4) SH-groups in bonito fish liver seems to be very unstable and affected (or influenced) by the lowering of the freshness as well as that in bloody meat. After the ground liver is incubated at 35°C. for 20 hrs., SH-groups are completely eliminated as shown in Fig. 4.

(5) It is assumed that the rapid decrease of SH-groups in bloody meat or liver may be probably observed on the process known as autolysis in which the values of volatile basic nitrogen are not so large.

The authors express their sincere gratitude to Prof. Dr. I. Tachi of the Kyoto University, for his invaluable advice throughout the course of the present study. We also wish to express hearty thanks to T. Oshida for this participation in the presnt study.

This study was supported in part by a Grant in Aid for Departmental Scientific Research from the Ministry of Education to which the offers their hearty thanks.

Literature cited

1) Okada, I., Osakabe, I., Kuratomi, Y. and Kikuchi, T.: J. Tokyo Univ. Fish. **39**, No. 1, 31–38 (1952).
2) Okada, I., Osakabe, I., Kuratomi, Y. and Sekine, E.: J. Tokyo Univ. Fish. **39**, No. 2, 186–191 (1953).
3) Kolthoff, I. M. and Lingane, J., James, J.: " Polaorography " 2nd ed., Vol. **2**, 858–862 (1952).
4) Mori, T. and Hata, M.: Bull. Japan Soc. Sci, Fish., **15**, 407 (1949).
5) Anson, M. L. and Mirsky, A. E.,: J. Gen. Physiol., **24**, 709 (1941).
6) Zuman, P.: Collection Czechoslov. Chem. Comuns., **16**, 510–525 (1951).
7) Kolthoff, I. M.: Stricks, W. and Morren L.: Anal. Chem. **26**, (1954).

Journal of the Tokyo University of Fisheries, Vol. 43, No. 2, 1957

POLAROGRAPHIC STUDIES ON THE CUTTLE FISH MEAT PART II

Isami Osakabe, Kiyoshi Fukushima, Takeaki Kikuchi and Ikunosuke Okada

(Received Feb. 11, 1957)

In former report (part I)[1], the authors found a well defined polarographic wave in the water extract of cuttle fish meat, and the constitutent showed reduction wave was recognized to be a certain organic base by the studies of details.

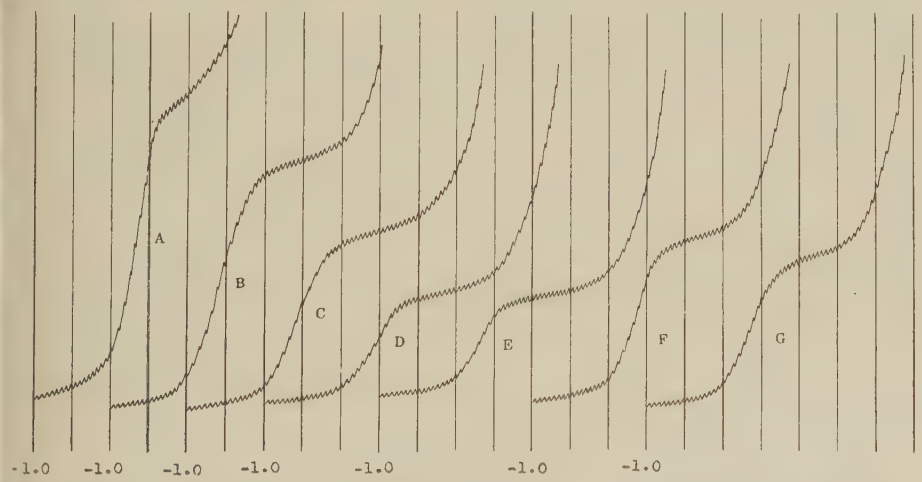
In this report the polarographic investigations of water extract of mollusca and crustacea meat were carried out, and the same polarographic wave was obtained in each sample as in the case of cuttle fish.

Since this substance showed reduction wave seemed to be closely related to serotonine (5-Hydroxytryptamine creatine sulfate monohydrate), the polarographic and spectrophotometric studies on serotonine and the extract of cuttle fish meat were carried out.

Experimental

I. Polarographic studies on the water soluble substances of mollusca and crustacea.

In this experiment the following samples were used; liver of cuttle fish, meat of dried cuttle fish, frersh meat of prawn, adducton of *Tapes Philippinarum*



Fig, 1. Polarograms of some molusca and crustacea meat. A, liver of cuttle fish, B, dried cuttle fish meat, C, adducton of oyster, D, adducton of *Tapes Philippinarum venerupis*, E, fresh meat of prawn, F, canned juice of *Erimacrus isenbekii* and G, canned meat of F. The supporting electrolyte was McIlvaine's buffer soln. of pH 7.0.

and oyster, canned meat and juice of *Erimacrus isenbekii*.

These meat of samples were homogenized with small quantities of water and diluted with McIlvaine's buffer soln. of pH 7.0 as 20 times, and the filrates were used to the polarographic electrolytic solution. These polarograms were shown on Fig. 1. In all samples well defined polarographic wave were obtained and the half-wave potentials of them were same as in case of cuttle fish meat. Since the reduction wave was obtained in the water extract of mollusca and crustacea, it will be widely distributed in invertebrate.

As this reduction wave was observed in the dried cuttle fish meat and canned meat and juice of *Erimacrus isenbekii*, so the substance showed reduction wave was assumed to be extremely stable.

II. Polarographic studies on serotonine.

The occurernce of serotonine in mollusca has been reported by Ersparmer[2], and Fujiwara[3], and the λ max of serotonine was 275 mμ, and it suggested the occurrence of serotonine in cuttle fish meat.

As reported in part I, the λ max of the organic base fraction which was separated from cuttle fish meat and showed a well defined polarographic wave was 272 mμ. As the λ max of the organic base fraction and serotonine are resemble in each other, polarographic and spectrophotometric investigations were carried out.

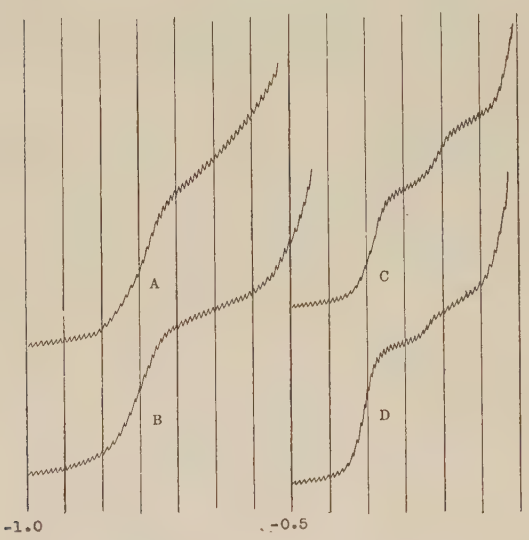


Fig. 2. Polarograms of serotonine and the organic base fraction from cuttle fish meat. A, serotonine and B, organic base fraction in McIlvaine's buffer soln. of pH 7.0, C, serotonine and D, organic base fraction in acidic soln.

Serotonine, used in this experiment, was given from Assist. Prof. Fujiwara of the Niigata University with his kindness.

These results were shown on Figs. 2 and 3. As shown on Fig. 2 (A, B) the reduction potential of serotonine in Mc Ilvaines buffer soln. of pH 7.0 was differ a little from the potential of organic base fraction separated from cuttle fish meat. The polarogram of each sample acidified with hydrochloric acid at pH>1.0 was shown on Fig. 2 (C, D). In each case the same polarographic wave was obtained that had two waves, and showed almost same potentials.

In the studies of the water soluble extract from the cuttle fish meat compared with serotonine by spectrophotometric method, the λ max of them were 272 mμ and 275 mμ respectively. These results were shown on Fig. 3. The difference of the λ max between themselves seemed to be caused by the same kind of other organic base which was involved in this fraction.

Consequentry, it was assumed that the substance showed the polarographic reduction wave in the water extract from cuttle fish meat or other mollusca and crustacea meat, was closely related to serotonine.

Further studies will be continued, when more quantities of serotonine is obtained.

Summary

1. The water soluble which was involved in cuttle fish meat and showed a well defined polarographic wave was considered to be widely distributed in invertebrates.

2. The substance showed reduction wave seemed to be closely related to serotonine.

Finally the authors express their hearty thanks to Assist. Prof. Fujiwara of the Niigata University, who was given the priceless serotonine for his kindness. We also wish to express hearty thanks to A. Onuki for his participation in the present study. This study was supported in part by a Grant in Aid for Departmental Scientific Research from the Ministry of Education to which the offers their thanks.

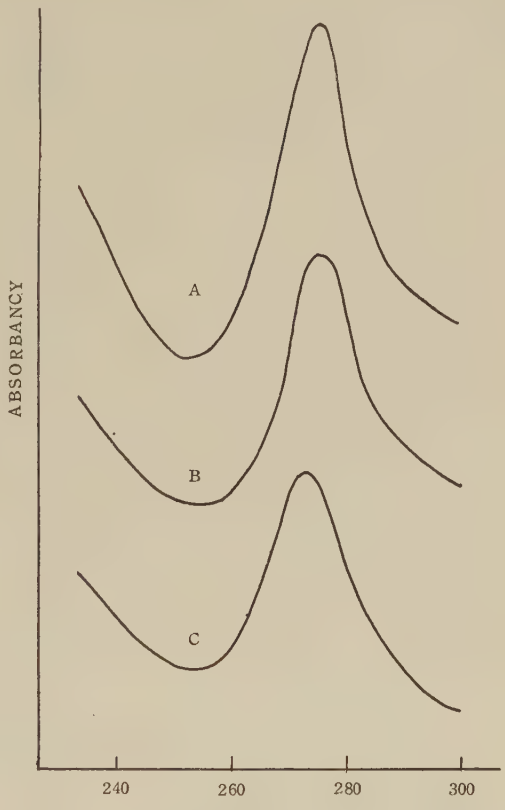


Fig. 3. Absorption spectrograms of serotonine and the organic base fraction from cuttle fish meat. A and B was serotonine in water and 1N H_2SO_4 soln. respectively, and C, organic base fraction.

References

1) OSAKABE et al: J. Tokyo Univ. Fish., vol. 34, No. 1 (1957).
2) ERSPARMER: Pharmacol review, vol. 6, p. 425 (1954).
3) FUJIWARA: Not printed (published at symposium on poisonous properties of cuttle fish).

Journal of the Tokyo University of Fisheries, Vol 43, No. 2, 1957

INVESTIGATION ON THE SOURCE AND AREA OF CONTAMINATION IN TOKYO BAY

Yutaka Sekine, Jun Takeuchi and Kunio Inoue

(Received Feb. 10, 1957)

Introduction

Tokyo Bay is a good fishing ground of the purple laver and the shell-fish, but now it has become the pool of sewage and dirty things from the cities surrounding it. As the bay is becoming dirtier day by day, we assume, the contamination is due to three causes.

1) As Tokyo and many other cities, those suddenly developed after the war are situated at the circumference of the bay. So these cities pour the sewage and dirts into it and the rivers contaminate it flowing through the cities.

2) According to the construction of Tokyo Harbour, the large ships set sail or anchor in the bay. So the contamination by these large and small ships form the great source.

3) By the development of the cities, the population is increased. So we can say that the main cause is the treatment of their faeces.

The former investigation of Tokyo Bay was thought from a biological point of view. So we carried out the hygienic bacterological investigation on the source and area of contamination in the Bay.

I. Procedure of Survey

In May and August we investigated the colony count and Coli form group[1] of the sea water at 37 stations in the bay, the Coli form group and the Enterococci of the purple larver and the shell fish. The state of the current in the bay is following Fig. 1.

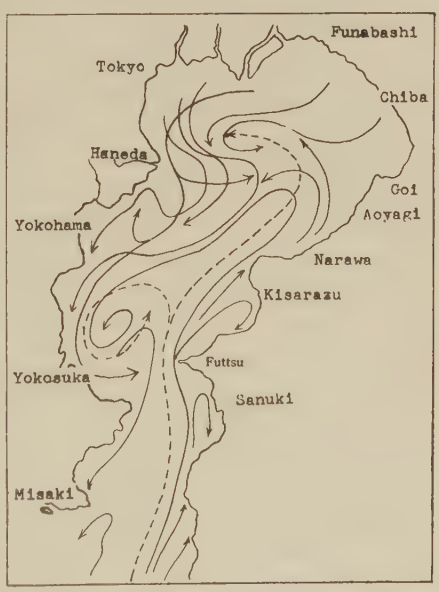


Fig. 1. The circulation of sea water in Tokyo Bay. (surface).
--→ main route of oceanic water.
→ main route of coastal water.

1) The water of the ocean that is to say a branch of the Japan current enters through the Uraga channel along the coast of Chiba and flows along Kannonzaki from Keihin district to the outside of the bay. Lately, there is a man who advanced the theory that a fairly strong current run contrary to it.

2) The investigation of contamination in the bay and the sphere of influence.

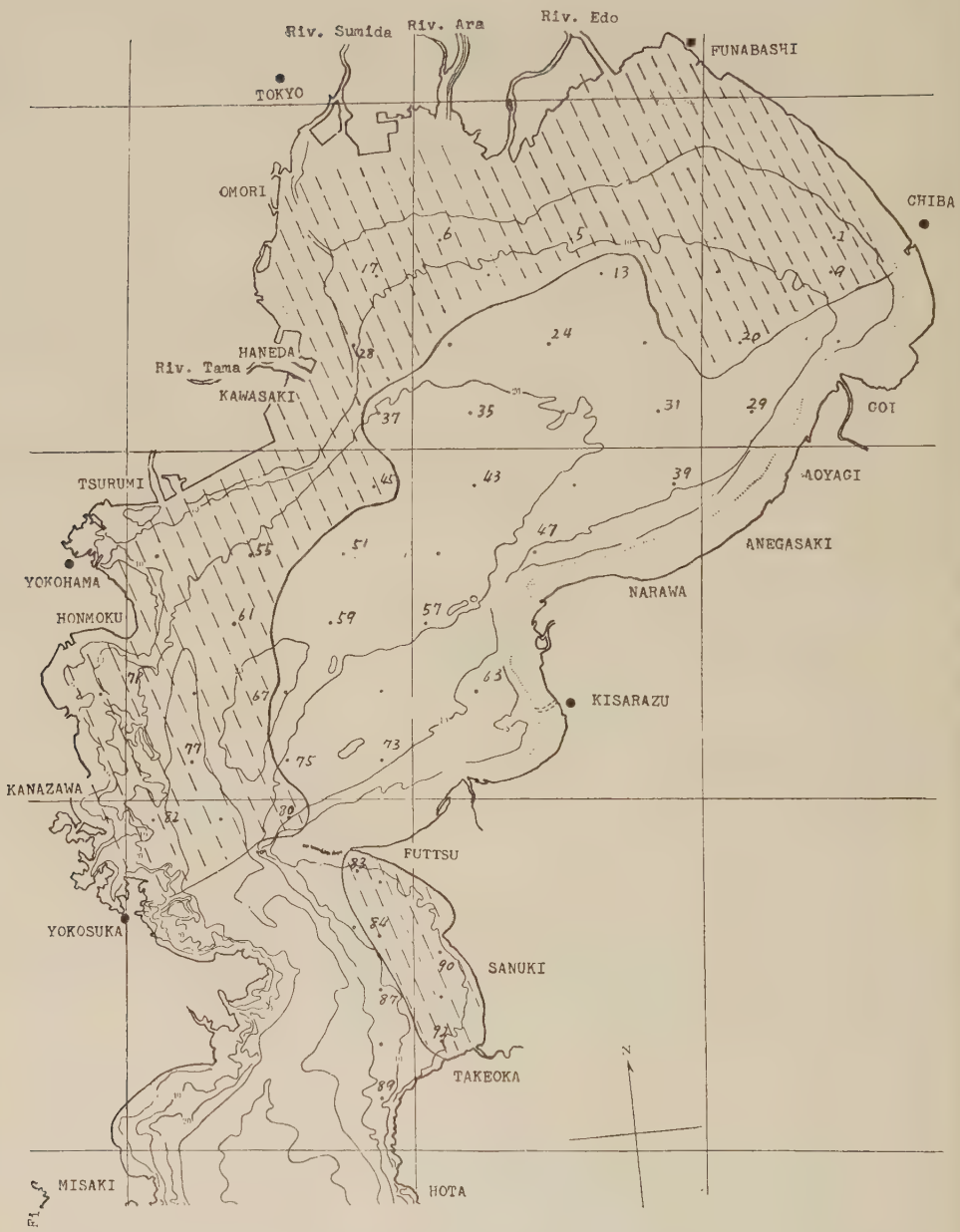


Fig. 2. Colony counts in sea water. (spring survey) from May 27 to May 31, water temp. 17–19°C. In the area covered by broken line, colony counts are over 50,000, and the numbers indicate the station.

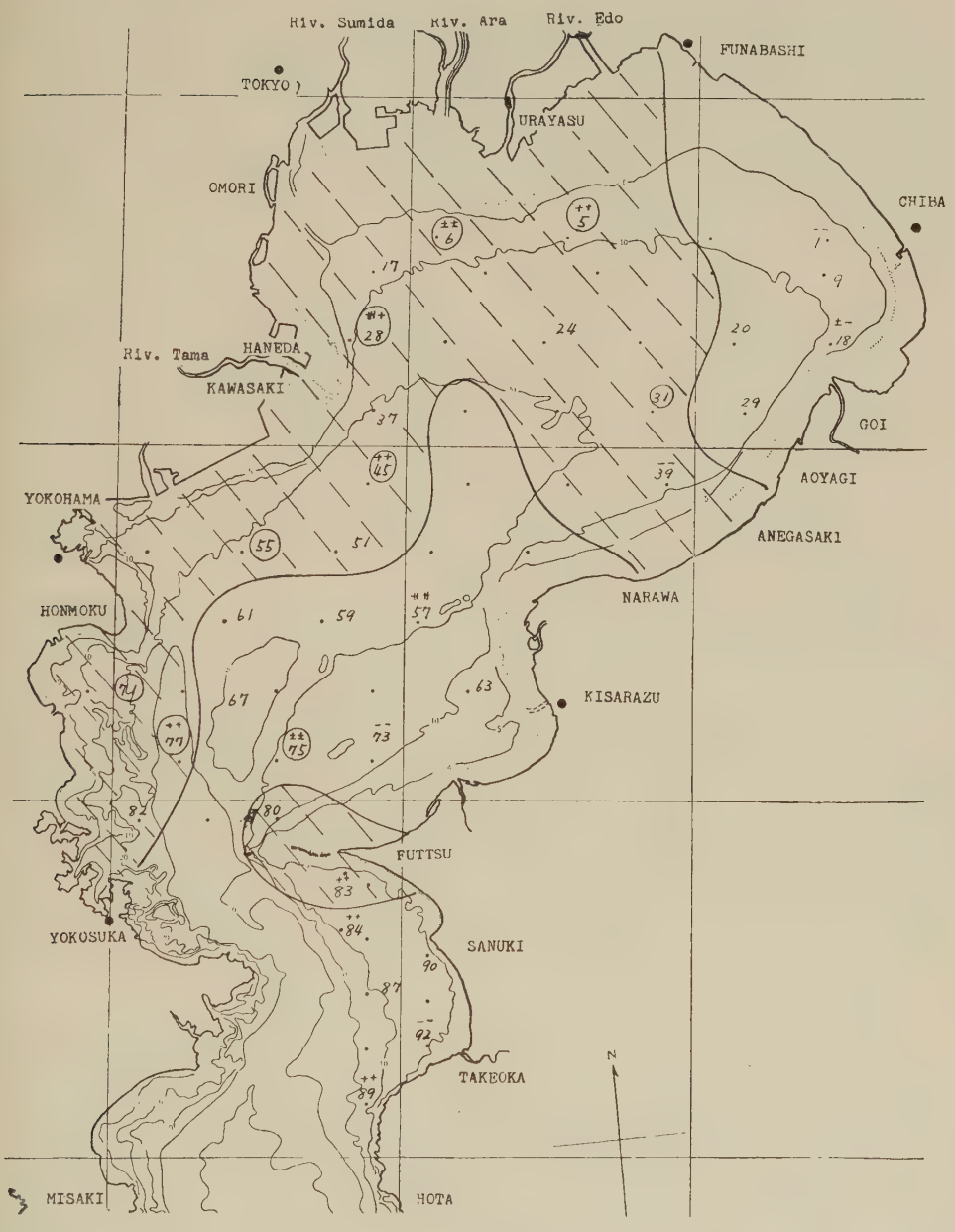


Fig. 3. Colony counts in sea water. (summer survey) from Aug. 30 to Sept. 5, water temp. 24-26°C. In the area covered by broken line, colony counts are over 100,000.

i) The state of contamination of sea water.

From a general hygienic point of view, the quantity of colony count becomes a standard for the judgement of the contamination. We selected 37 stations in the bay and investigated colony count, Coli form group M. P. N. and Enterococci M.P.N. of the surface water in spring and summer. The procedure we follwed in that the culture media were carried in the ship and as soon as the water and the mud were collected, we cultured them. The work after that was carried out in our laboratory.

(1) Colony count in the sea

i) The investigation in spring (1955, May 27–31).

The collected sea water was diluted with 0.85% NaCl. Then 1.0 cc. of the sol. was cultured at 35–37°C in conformity with the drinking water guide[1)], got all the averages of the deep colony number. The result is shown in Fig. 2.

Generally, the water district of north and west coast, from 2.5 to 3.5 miles had colony count of over 50 thousand.

ii) The investigation in summer (1955, Aug., 30–Sept., 5.)

In summer the colony count was 20 to 30 times more than that of spring due to the heavy rain and high temperature (24–27°C). The result is following Fig. 3.

Considering from this result, it makes the normal form without much rains in spring. For example, the colony counts are few at the east coast and many at the west coast. The east coast is clearer than the west coast.

The reasons are; 1) the east coast becomes the passage of the Japan current, 2) on the west coast the great cities are many and the inflowing rivers are many at the north and west coast, 3) the water district that is indented from the south of Futtsu-misaki has a great many of colony count for the sewage stay on the coast of the indented bay. Near these places the faeces are thrown and when the current goes up to the north by the open sea water, it stays there. 4) In summer, the river swells due to the heavy rain and it flows to the bay, down to the south and the influence of the river appears at the centre of the bay.

(2) Coli form group M.P.N, th the sea water.

i) The investigation in spring

The investigation was carried out as to the colony count and the Coli form group M.P.N., by collecting water at the spot and afterwards observed in the laboratory. The result of the investigation in spring is shown in Fig. 4. The positive district of Coli form group is 4 or 5 miles off the land along the north west coast from Ichikawa to Tomioka in Kanagawa district. And a part of it showed positive at an observation station No. 90 at Sanuki. At the station from Haneda to Yokohama it proved the presence of E. coli type. At some special stations, specific observation of HNO_2–N and NH_3–N in water were tested qualitatively and it showed all positive results.

ii) The investigation in summer

It is like the investigation in spring as Fig. 5 and the positive district of Coli form group was admitted at 2 or 4 miles off the west coast from Funabashi

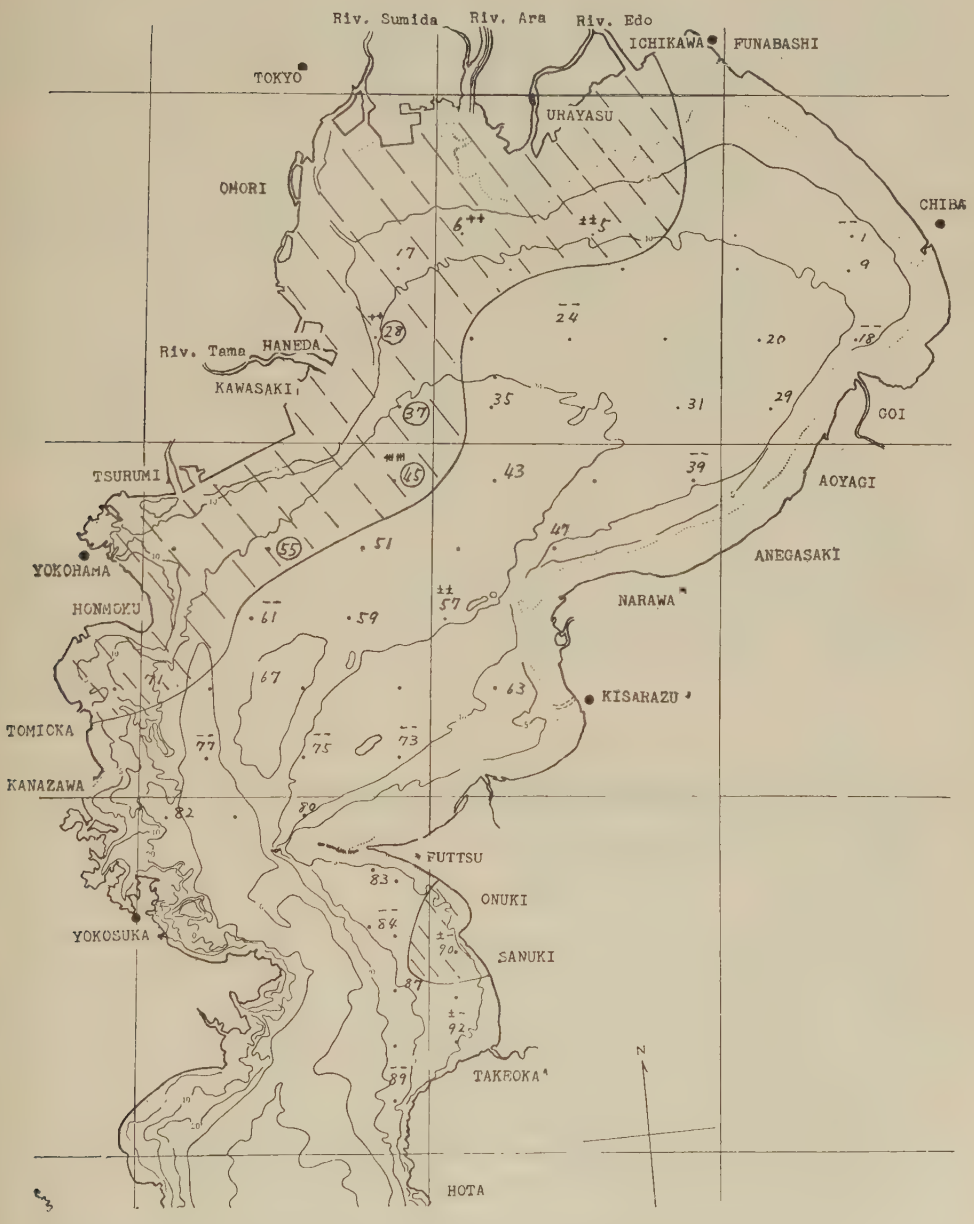


Fig. 4. M.P.N. of Coli form group in sea water (spring survey).

1) In the area covered by broken line, M.P.N. are over 100.
2) Nitrite-N and ammonia-N are indicated by ++, the left side of (++) indicated the result of HNO_2-N and the right side is NH_3-N.
3) At the station number closed by circle, E. coli was detected.

Fig. 5. M.P.N. of Coli form group in sea water, (summer survey)
In the area covered by broken line M.P.N. are over 100.

to Yokosuka. It showed the extensive positive district of Coli form group from the west of Funabashi to Narawa. And at the surrounding of Futtsu it showed the positive district. E. coli type masses the station from Yokohama to Kanazawa, HNO_2–N and NH_3–N are positive at the water districts of the Edo and the Ara and on the west coast.

In spring the result of M.P.N. is the same with the colony count, showed positive at the north west coast and all negative at the east coast. On the station off the coast of Sanuki it was admitted the same contamination of water district, as well as in colony count.

In summer also the contamination is as well as in colony count on the west coast and extended from the coast of Anegasaki to that of Narawa according to the swelling of the Edo and the Ara. The water district of contamination was admitted at the circumference of Futtsu same with the investigation is spring. Coli in the sea water generally belongs to Aerobacter and E. coli, was mainly present in the dirty west coast.

iii) The state of contamination at the bottom (Coli form group M.P.N.)

The bottom is almost mud and the south of the sand-bank in the centre of the bay is almost sand. There is no difference in spring and summer and was regularly positive along the coast to a long distance, say 2–8 miles. The muds are generally the Coli form group positive and so not change by the season as the sea water. Even if the parts of the sand or the gravels are positive, the M.P.N. is very low. The colony count at the bottom is very much, differing from the sea water.

(3) The investigation of shell fish

i) Coli form group M.P.N. of *Asari*.

We investigated the shell fish at eight nurseries. The bottom was all mud but that of the south of Tomioka and the east and the west coast was more or less mixed sand. We carries on the collection almost at the ebb time. There was not much difference in the M.P.N. in eight stations as shown in Fig. 6, but the M.P.N. is more or less low at Urayasu. On the east coast i.e. Chiba it showed only 10^5, but others are all more than 10^4 and are very dirty. On the west coast i.e. Kawasaki, Honmoku and Kanazawa showed a high value, thrice.

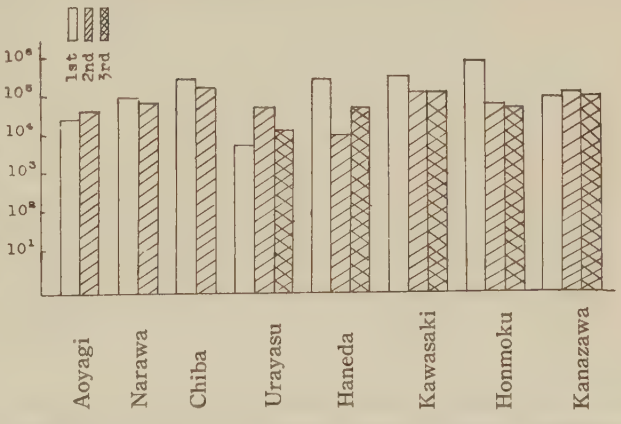


Fig. 6. M.P.N. of Coli form group in clam (*Venerupis semidecasata*). (from Nov., 54 to Jan., 55).

ii) Enterococci M.P.N.

When it is compared with the former it showed pretty low value. At

Aoyagi it showed negative twice and at Narawa it is very low, that is ten or so. Kawasaki, Honmoku and Kanazawa are dirtier than the others and the degree of contamination on the west coast is high. If Enterococci is compared with Coli form group, the rate of finding is low (Fig. 7).

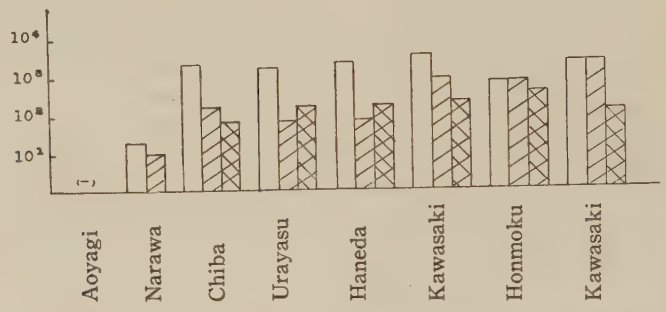


Fig. 7. M.P.N. of Enterococci in clam (*V. semidecasata*). (from Nov., 54 to Jan., 55).

iii) Coli form group M.P.N. of hard clam

Japanese hard clam at Urayasu is comparatively low as the former but that at others stations is higher having not much difference among them (Fig. 8).

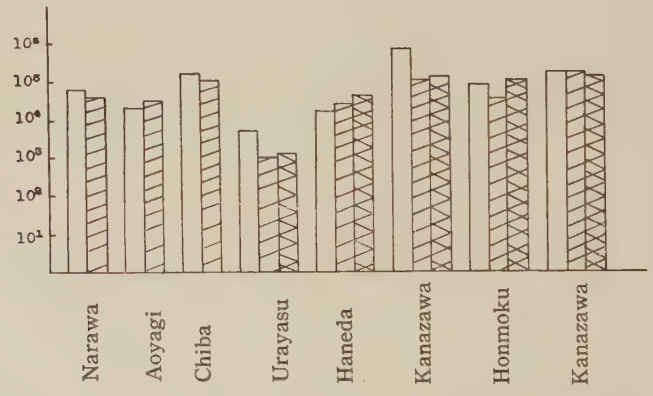


Fig. 8. M.P.N. of Coli form group in clam (*V. semidecasata*). (from Nov., 54 to Jan., 55).

On the whole, Kawasaki, Honmoku and Kanazawa on the west coast are comparatively dirtier than the places on the east coast.

iv) Enterococci M.P.N.

If Enterococci is compared with Coli form group the rate of positive is very low as mentioned above and the station at Aoyagi was entirely negative. The Enterococci M.P.N. on the west coast showed a high degree, compared with the east coast (Fig. 9).

The degree of contamination of bloodly clam (Baka-gai) on the west coast

is very high with *Asari* and Japanese hard clam, and particularly that at Kanazawa showed a high value.

v) Coli form group M.P.N. of (Baka-gai)

According to the differences seen at Honmoku and Aoyagi, Aoyagi is low and the rest were more than 10^3 (See Fig. 10).

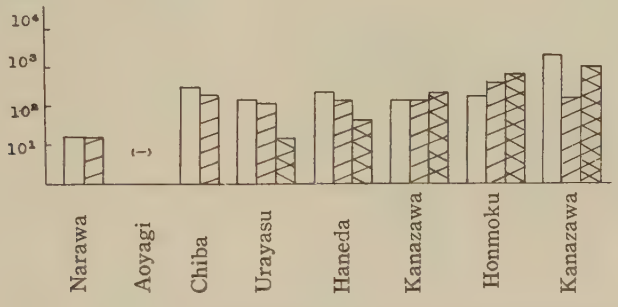


Fig. 9. M.P.N. of Enterococci in clam (*V. semidscasata*).

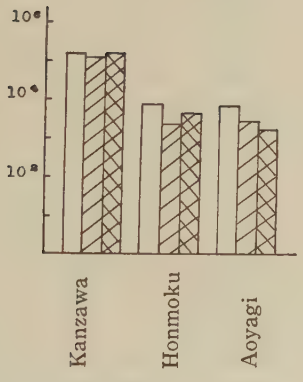


Fig. 10. M.P.N. of Coli form group in bloody clam (*Anadara* sp.).

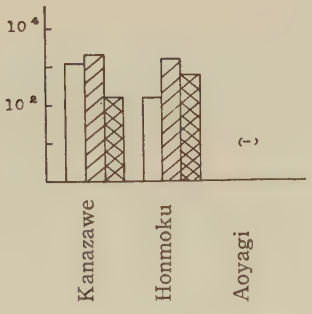


Fig. 11. M.P.N. of Enterococci in bloody clam *Anadara* sp.).

vi) Enterococci M.P.N,

The result as to the Enterococci is the same and the station at Aoyagi showed negative thrice. The two others i.e. Honmoku and Kanazawa though positive, yet the Enterococci M.P.N. is lower than the Coli M.P.N. but this showed a high value at west coast (See Fig. 11).

vii) Coli form M.P.N. in sea water and mud of shell fish nurseries.

We investigated Coli form group M.P.N. in the sea water and in the mud of eight shell fish nurseries.

The sea water showed no difference with the bottom-mudlike the above graph, but the mud showed a little high value than the sea water except at Narawa. Comparing all, the result of water examination is like that of the shell fish M.P.N. in the sea water and the mud at Urayasu is comparatively low. It is clearly appeared that all the east coast is clear and the west coast is dirty.

viii) Enterococci M.P.N.

If we observe Enterococci, the sea water at the east coast is negative and that at Urayasu is negative, too. On the contrary, the west coast is positive of 10 or so (Fig. 13).

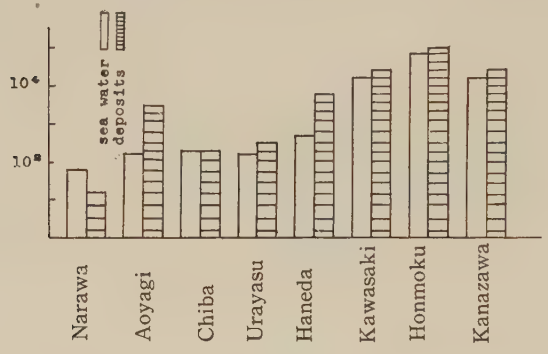


Fig. 12. M.P.N. of Coli form group in sea water and bottom deposits (mud).

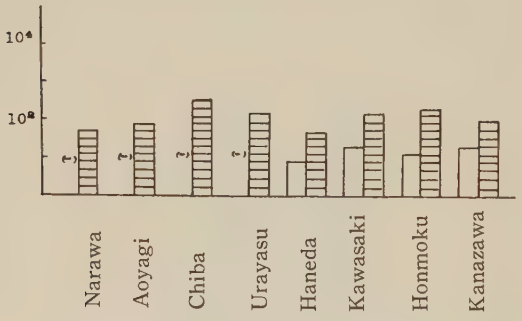


Fig. 13. M.P.N. of Enterococci in sea water and bottom deposits (mud).

The mud of the eight nurseries showed positive having equal degree. The result of that is very different from the sea water.

ix) Coli form group of oyster

We investigated the oyster at Haneda, Kawasaki, Funabashi and Goi. The Oyster at Haneda is the natural, at Kawasaki, Funabashi is Hanging by stick and that at Goi is Hanging method, it is well known fact that the way of oyster culture by Hanging method is the most rational and hygienic way. This truth shows clearly and that of Goi is the lowest and the next one is Hanging by stick at Funabashi. Hanging by long line at Kawasaki is inferior to seeding method at Haneda. Generally speaking, we wonder if this has no influence on the way of culturing oyster on account of contamination at the

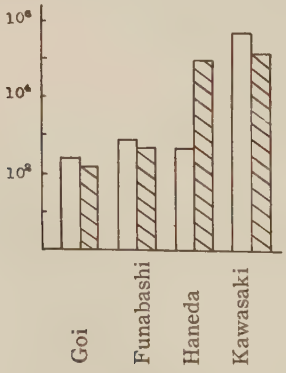


Fig. 14. M.P.N. of Coli form in oyster (*Ostrea* sp.) (from Oct., 55 to Nov., 55).

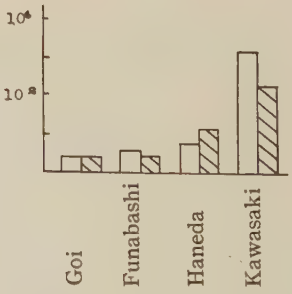


Fig. 15. M.P.N. of Enterococci (*Ostrea* sp.).

west coast is high. But an ivestigation on perpendicular distribution is going on at present. The result of investigation of the distribution at the mouth of Tokyo Bay carried out at Misaki Health centre[1] showed high value at the surface and low value at the lower layer (Fig. 15).

x) Enterococci M.P.N.

Compared with the former this is very low. And as the former the Hanging method at Goi showed the lowest value and Hanging by long line at Kawasaki showed the highest value.

xi) The bottom (mud) M.P.N.

The bottom always showed high value, compared with the sea water. The difference between both the coasts is not remarkable, but the east coast is more or less low and the west is high.

xii) The sea water (Enterococci M.P.N.)

At Goi and Funabashi it showed negative twice but at Haneda and Kawasaki though it showed low value yet it is positive. Comparing these two, it showed high degree of contamination at Kawasaki (Fig. 17).

xiii) The bottom (mud) (Enterococci M.P.N.)

The mud showed positive but at Goi it showed the lowest degree and at Kawasaki it was the highest. According to this, the result of oyster is the same as that of the mud.

xiv) HNO_2–N, NH_3–N in sea water at oyster nursery.

From the result of the water investigation, the state of contamination can be judged like the former (Table 1).

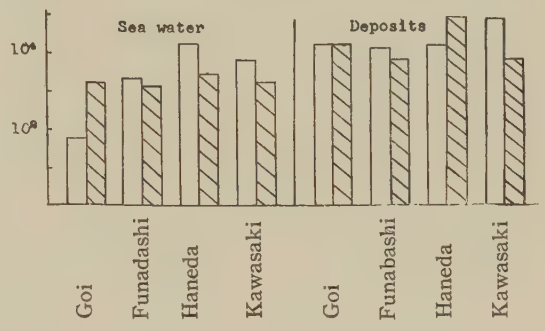


Fig. 16. M.P.N. of Coli form group in oyster culture ground.

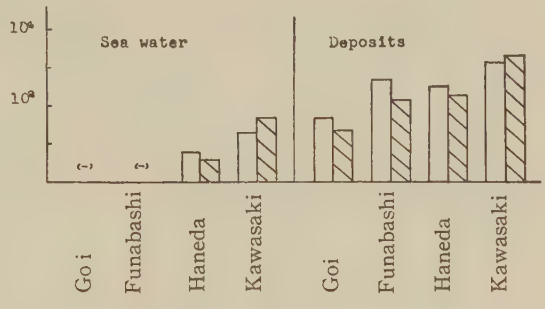


Fig. 17. M.P.N. of enterococci in oyster culture ground. (from Nov., 55 to Dec., 55).

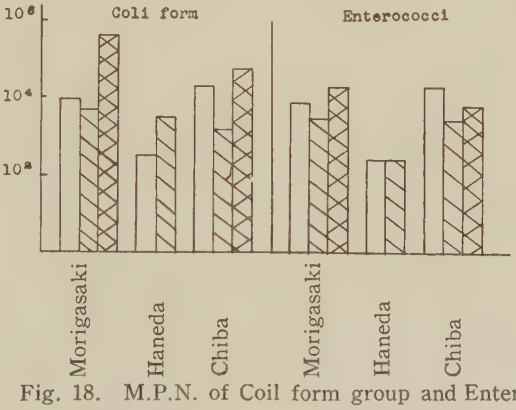


Fig. 18. M.P.N. of Coil form group and Enterococci in *Porphyra*.

Table 1. Water examination of oyster cultural ground.

Cultural ground	HNO_2–N		NH_3–N	
	I	II	I	II
Goi	+	+	+	+
Funabashi	++	±	+	+
Haneda	+++	++	++	+
Kawasaki	+	++	++	+

(4) The investigation of purple lavers

In March two or three times we investigated them, collecting at Morigasaki, Haneda, Chiba and so on. Comparing the three purple lavers, those at Haneda were comparatively good and those at Chiba and Morigasaki were next to it.

We found that the contamination of purple laver showed fairly a high degree and its colony count per gram was from about ten thousand to about hundred thousand. As Enterococci showed all high degree of contamination than the shell fish. Originally the purple lavers have a great deal of bacteria and the mucus of the seaweeds becomes a good factor for the growth of it (Fig. 18, Fig. 19).

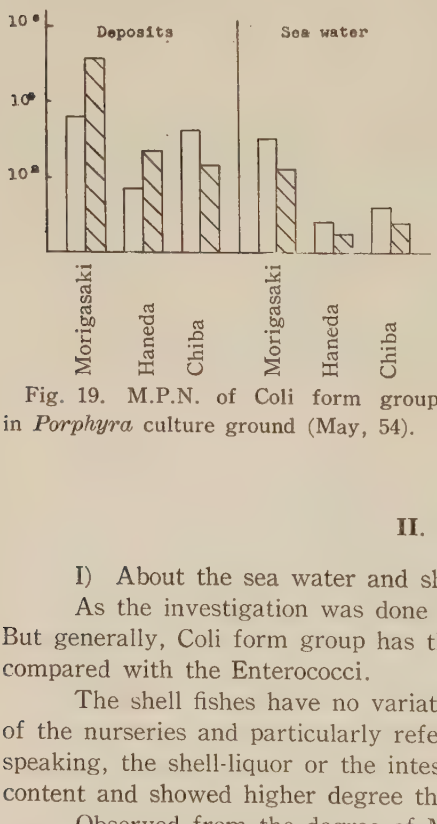


Fig. 19. M.P.N. of Coli form group in *Porphyra* culture ground (May, 54).

As to both bacteria group M.P.N. of the sea water in the purple lavers nursery, we can find the difference. The degree of contamination was low at Haneda and highest at Morigasaki. I wonder whether the comparative high degree showed at Chiba is due to the influence of the Riv. Miyako flowing through the city of Chiba. On all the occasions, Enterococci showed lower degree than Coli form group.

II. Summary

I) About the sea water and shell fish

As the investigation was done only twice or thrice, the result is overhasty. But generally, Coli form group has the high rate of finding on all the occasions, compared with the Enterococci.

The shell fishes have no variations, but they depend on the circumstances of the nurseries and particularly refer to the mud at the living place. Generally speaking, the shell-liquor or the intestines of the shell fish have always bacteria content and showed higher degree than the sea water and the mud.

Observed from the degree of M.P.N. in both the bacteria group, the east coast is clearer than the west coast.

II) About the purple lavers.

Of course, the purple lavers that settle in the water are influenced by the degree of contamination of the sea water in the nursery, differing from the shell fish that live in the mud. At the conjested place of the laver, the water does not flow and naturally stagnant. Therefore, when the purple laver is cultivated in the contaminated sea water, it is sure that the contamination is higher due to the sticking of large number of bacteria as written before.

III) The degree of contamination in the main rivers pouring into the bay.

As the main cause of contamination in the bay the rivers pouring into it, we tried to investigate the degree of contamination of these main river—the river of Tama, Sumida, Ara and Edo. Three stations were selected at a distance of ten kilometers in the upper, middle and down stream from the mouth for collecting water for the investigation as thown in Fig. 20. As usual, the qualitative test for the Coli form group Enterococci and water was caaried out.

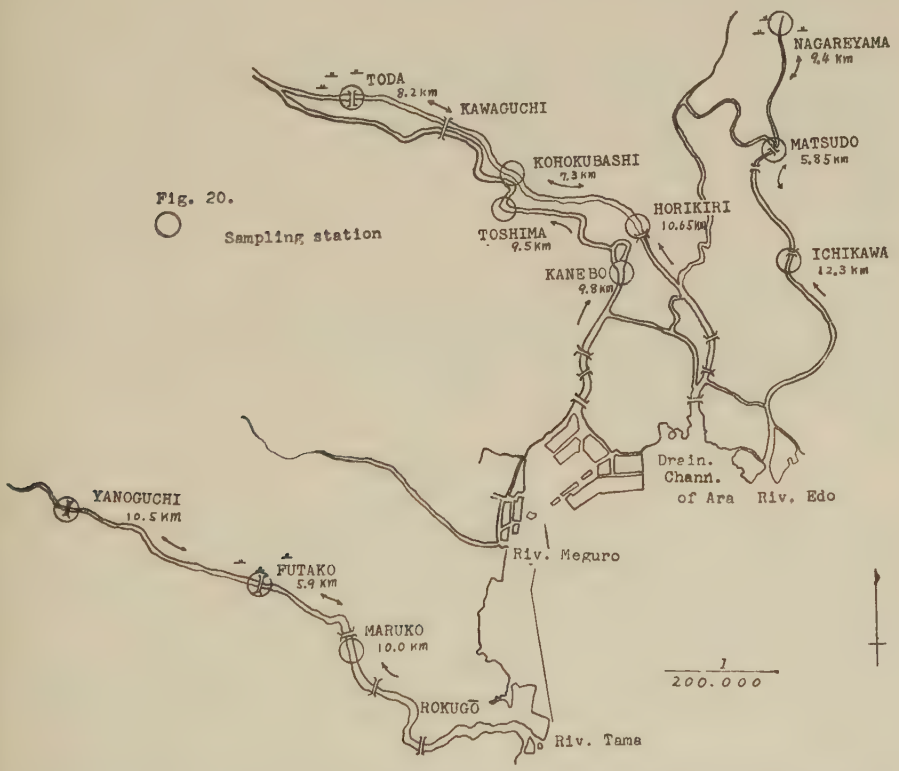


Fig. 20. ○ Sampling station

As we proceed in the down stream in every river (Table 2) Colony count Coli form group and Enterococci M.P.N. increased. The same case is with HNO_2-N and NH_3-N. The Sumida had the highest degree of contamination among the four rivers. Coli form group M.P.N. was unlimited at both its middle and down stream Enterococci M.P.N. showed minimum 2,400. By the qualitative test of the water they showed severe positive at middle and down stream. The river

having the comparatively low degree of contamination was the Tama and its NH_3-N showed all negative. The water collected in the down stream was brown or opaque white and particulary that of Sumida was very muddy. The station for collecting water in the down stream was ten kilometers from the mouth, but

Table 2. Contamination degree of main river flowing into Tokyo Bay

River	Riv. Tama			Riv. Sumida	
Sampling station	Yanoguchi	Futako	Numabe	Toyoshima	Kanebo
Colony count	6×10^3	10×10^3	16×10	10×10^3	48×10^3
Coli M.P.N.	5×10^3	32×10^3	18×10	∞	∞
Entero. M.P.N.	2×10^2	2×10^2	8×10^2	24×10^2	84×10^2
HNO_2-N	+	++	+++	++	+++
NH_3-N	−	−	−	++	+++

River	Riv. Ara			Riv. Edo		
Sampling station	Toda	Kohoku	Horikiri	Nagareyama	Kasai	Ichikawa
Colony count	2×10^3	2×10^3	8×10^3	3×10^3	12×10^3	18×10^3
Coli M.P.N.	14×10^3	50×10^3	54×10^3	16×10^3	24×10^3	73×10^3
Entero. M.P.N.	12×10^2	54×10^3	72×10^2	37×10^2	54×10^2	54×10^2
HNO_2-N	+	+	+	−	−	+
NH_3-N	+	+	+	±	−	−

this distance along the bank is crowded with houses and factories. On account of this these river was highly contaminated in this short distance, we anticipate the contamination of the river will increase more in future due the tendency to build more houses in that area.

III. Conclusion

1. Both the sea water and the living things are clean at the east coast that is the passage to the open sea water compared with the west coast.

2. The source of contamination in the bay is mainly the pouring rivers. We cannot overlook that one of the high degree of contamination of the west coast is by the large ships.

3. Around Futtsuzaki and on its south coast the water district of contamination was found locally due to the accumulation of the sewage and faeces is projecting.

4. Enterococci has generally lower rate of finding than Coli form group. Coli form group is E. coli I type, in addition to the Aerogenes type and the Intermediate type. For this reason the finding of the Coli form group is high.

As the Enterococci is the faecal type, it can be very well used as an indicator for contamination than the Coli form group.

In short, the degree of contamination of fisheries stock in Tokyo Bay is very high by the contamination in the Bay, but the water district in east coast

which is comparatively clean is the good fisheries ground.

As to the high degree of contamination of the purple laver, if the stagnant water flows continuously then a fairly good result can be obtained. For the shell fish, artificial purification should be carried out.

At the end we wish to express our heartfelt thanks to the Tokai Regional Fisheries Research Laboratory and the Fisheries Experimenting stations of Tokyo, Chiba and Kanagawa for the help extended to us. The investigation was carried out from the scientific expenses of the Ministry of Education.

References

1. Welfare Ministry 1950. Hygienic Examinations Guide IV. Drinking water Examinations Guide.
2. ——— 1951. Hygienic Examinations Guide III. Food Hygienic Examinations Guide.
3. Litsky, W., Mallmann, W. and Fifield, C. W. 1943. New medium for the detection of Enterococci in water. Americ. J. Pub. Health. 2, pp. 673–879.
4. Misaki Health Centre at Kanagawa District 1955, Dec. Studies on the influence and the investigation of the contamination throwing the faeces in the seas according to the current at the mouth of Tokyo Bay and Sagami Bay.

Journal of the Tokyo University of Fisheries, Vol. 43, No. 2, 1957

ATMOSPHERIC EXPOSURE OF NETTING THREADS FOULED WITH FATTY ACID, LINSEED OIL AND SARDINE OIL

Katsuji Honda, Takeaki Kikuchi and Ikunosuke Okada
(Received Feb. 11, 1957)

Introduction

Fish nets are apt to be foul with fat, mucus and blood of fish while in use. Usually fish nets are throughly washed and they are dried by exposure to the sun after use and then they are stored. When a fish net foul with some of the above mentioned substances is dried by exposure to the sun, it is supposed to be affected by ultraviolet rays and its life to be shortened.

Formerly S. Doi[1:63] reported that when cotton, vinylon and amilan netting threads were saturated with shark liver oil and then they were hung in the thermostat keeping 30°C R.H. 100%, amilan was affected by that oil but the others were hardly affected.

Tôyô Rayon Co.[1:60] reported that when cotton and amilan netting threads were soaked into cod liver oil and then they were hung in the thermostat keeping 40°C R.H. 100%, they were affected by that oil.

I. Okada and others[2] reported the diminution of strength on cotton netting threads faul with fatty acid and fish oil by exposure to the sun.

In the present paper the authors studied the effect of exposure to the open air of various netting threads treated with palmitic and oleic acid as the reprentatives of saturated and unsaturated fatty acids, linseed and sardin oil as that of dry and fish oil respectively.

Experimental

I. Material and method

Details of materials are shown in Table 1. The threads which were treated by the following method were cut about 1.2 meters respectively and fastened to two iron frams. These samples were exposod on the roof facing south of the

Table 1. Details of netting threads.

Material	Construction	Tensile strength kg
Cotton	20S/15 Z	10.8
Cremona	18S/18 〃	12.6
Kanebian	20S/15 〃	12.2
Saran	260D/18 〃	17.5
Amilan	250D/12 〃	10.8

Tokyo University of Fisheries at Kurihama, keeping angle of about 45 degrees to the horizon, from July 5, '54 to Jan. 1, '55.

At the end of every month during our experimental period, every 10 threads

from the samples were unfastened from the two iron frames and tested for tensile strength. These tests were always carried out under the dry condition of threads.

II. Symbol and treatment

A White thread—untreated—used as a control.

B Coal tar—The threads were soaked into heavy tar (not diluted with creosote) for 30 minutes at 80°C, used as a comparison.

C Oleic acid—The threads were treated in oleic acid for 30 minutes. After the threads had been drawn out of the oleic acid they were drawn between the fingers in order to remove the excess oleic acid adhering to the threads. The properties of oleic acid were, Iodine value 72.3, Neutral value 231.5. It seemed to suffer a slight oxidation.

D Palmitic acid—The threads were treated in saturated palmitic acid in petroleum-ether for 30 minutes. Melting point of this palmitic acid was 62.0°C.

E Linseed oil—The threads were treated in saturated linseed oil for 30 minutes.

F Sardine oil—The threads were treated in sardine oil for 30 minutes. These symbols and threads treated with above substances were shown in Table 2.

Table 2. Symbol and treatment.

Symbol					Treatment
Cotton	Cremona	Kanebian	Saran	Amilan	
C_1	K_1	N_1	S_1	A_1	White thread, untreated, control
C_2	K_2	N_2	S_2	A_2	Coal tar
C_3	K_3	N_3	S_3	A_3	Oleic acid
C_4	K_4	N_4	S_4	A_4	Palmitic acid
C_5	K_5	N_5	S_5	A_5	Linseed oil
C_6	K_6	N_6	S_6	A_6	Sardine oil

III. Wheather condition at Kurihama

The temperature, humidity, cloud and total insolation duration during the period of this experiment are shown in Table 3. Each value except that of insolation duration is the mean value during those 30 days.

Table 3. Weather condition at Kutihama.

Period	Temperature °C	Humidity %	Amount of clouds	Insolation duration hour
July 5 — Aug. 3	22.1	84.2	8.3	119.8
Aug. 4 — Sept. 2	26.8	80.9	6.7	207.6
Sept. 3 — Oct. 2	23.8	81.3	6.3	189.4
Oct. 3 — Nov. 1	15.3	77.1	7.4	106.1
Nov. 2 — Dec. 1	11.6	71.4	5.4	160.8
Dec. 2 — Jan. 1	7.3	63.1	4.2	141.1

IV. Tensile strength

The figures given to all the threads are the mean values of 10 tests on the

threads of 20cm in span length, under dry condition. The tensile strengths of the initial stage and after some days passed are denoted by T_0 and T respectively. Table 4 gives the results of measurements of the tensile strength and T/T_0 of

Table 4. Values of T/T_0 on netting threads exposed to the open air at Kurihama '54–'55.

Symbol	T_0 Unexposed	T/T_0 Days exposed					
	(kg)	30	60	90	120	150	180
C_1	10.8	.59	.47	.39	.40	.39	.39
C_2	12.1	.59	.59	.59	.81	.92	.95
C_3	12.3	.53	.05	.08	.07	.16	.08
C_4	11.9	1.05	.46	.44	.37	.47	.45
C_5	12.1	.63	.14	.13	.14	.17	.14
C_6	11.7	.63	.10	.13	.10	.10	.14
K_1	12.6	1.20	.60	.40	.55	.50	.49
K_2	12.5	1.08	.63	.71	.68	.59	.68
K_3	11.1	.98	.46	.36	.33	.39	.44
K_4	11.9	1.25	.52	.56	.41	.37	.39
K_5	11.2	.93	.52	.50	.56	.39	.52
K_6	12.7	.88	.41	.44	.43	.40	.37
N_1	12.2	1.00	.44	.45	.41	.38	.36
N_2	11.9	.74	.42	.59	.56	.57	.53
N_3	11.8	.61	.21	.23	.16	.23	.21
N_4	12.2	.84	.47	.43	.41	.36	.37
N_5	11.8	.65	.20	.28	.23	.22	.37
N_6	11.6	.55	.19	.27	.17	.23	.22
S_1	17.5	.69	.37	.37	.26	.25	.25
S_2	17.0	.95	.50	.56	.58	.56	.64
S_3	17.9	.77	.34	.25	.25	.34	.27
S_4	20.2	.72	.32	.23	.26	.24	.26
S_5	19.0	.42	.31	.29	.28	.30	.31
S_6	19.0	.33	.34	.31	.30	.29	.32
A_1	10.8	1.29	.45	.50	.20	.53	—
A_2	11.2	1.35	.59	.68	.67	.59	.74
A_3	11.1	.96	.22	.16	.15	.18	.21
A_4	11.5	1.04	.46	.33	.13	.22	—
A_5	11.2	1.06	.16	.13	.08	.14	.15
A_6	11.0	.81	.08	.12	.08	.11	—

the threads exposed to the open air at Kurihama. The same results are expressed graphically in Figs. 1 and 2. Figure 1 shows the results of the tests on every thread and Figure 2 as to every substance.

V. Results

Generally, the threads both untreated and treated tend to be weakened by atmospheric exposure. The tensile strength showed the utmost decrease in July, Aug. and Sept. having most sun hours.

The strengths of the thread A, E and F were reduced to less than 50% of the initial stage at the end of Sept. Above all, the threads faul with sardine oil F suffered remarkable diminution of strength, but all fatty materials here used showed only trifling differences among themselves. The threads treated

with coal tar were not remarkable than the others in diminution of tensile strength. From the above fact, it seemed that the surface of fiber was coated with black colored free carbon in coal tar, which intercepted the effect of ultra violet rays.

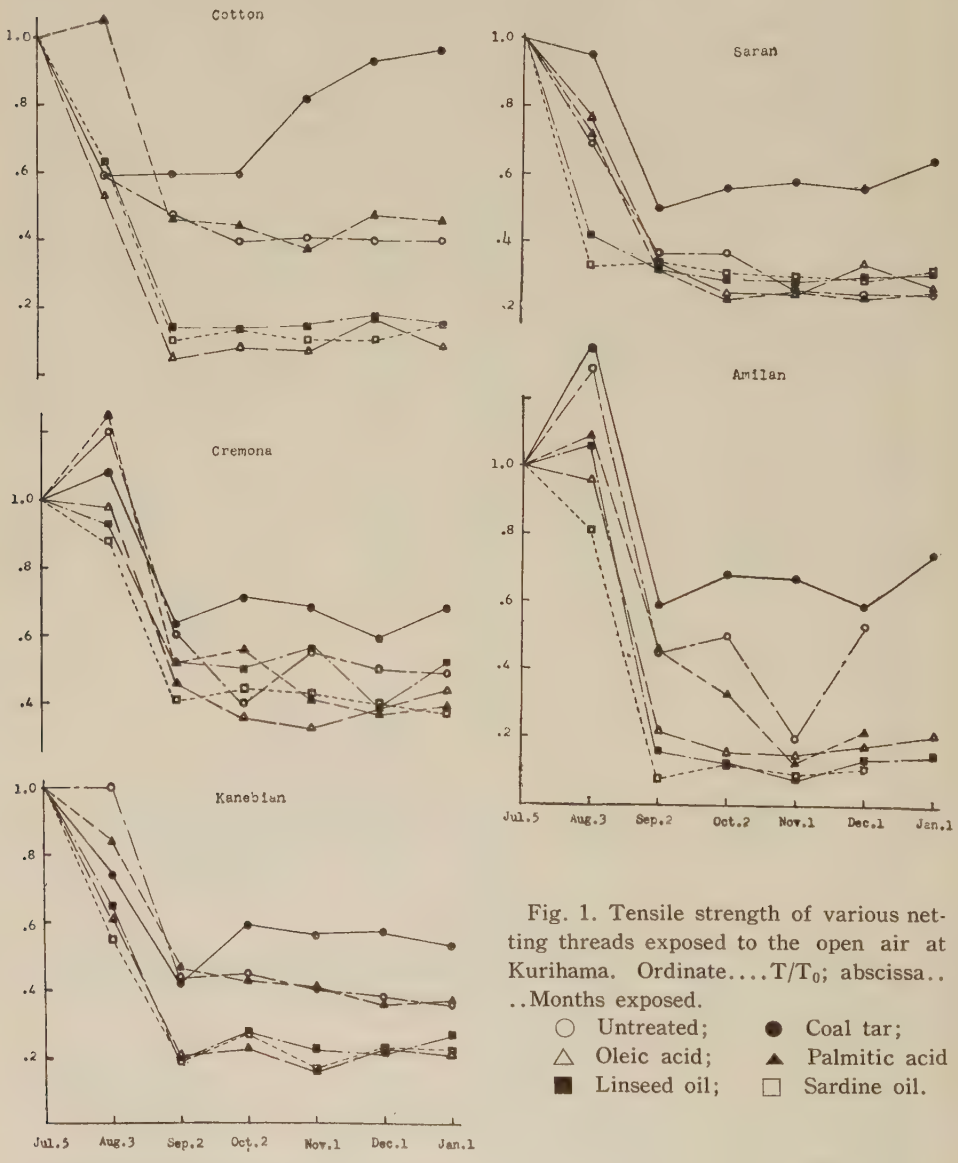


Fig. 1. Tensile strength of various netting threads exposed to the open air at Kurihama. Ordinate....T/T_0; abscissa....Months exposed.

○ Untreated; ● Coal tar;
△ Oleic acid; ▲ Palmitic acid
■ Linseed oil; □ Sardine oil.

I) On each thread

A. Cotton

Cotton threads treated with oleic acid, linseed oil and sardine oil were remarkably affected by exposure to the open air and their strength became about

15% of the initial stage after two months (July, August) and thereafter kept nearly the same value as that. The treatment with palmitic acid was better than the above and its strength became about 45% of the initial stage after two months and thereafter was hardly weakened.

The control (untreated) followed almost the same as the course of palmitic

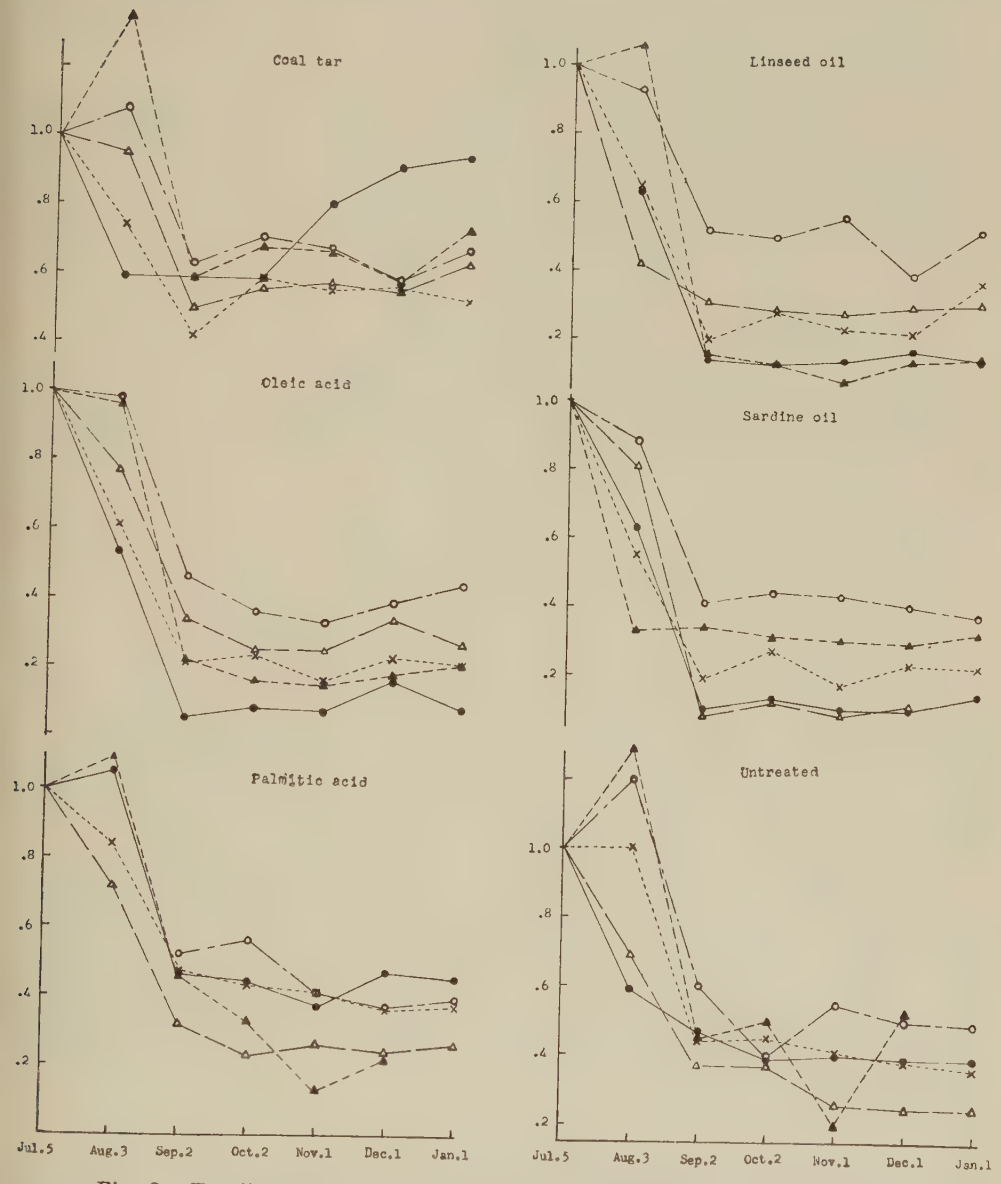


Fig. 2. Tensile strength of various netting threads exposed to the open air at Kurihama. Ordinate....T/T_0; abscissa....Months exposed.

●, Cotton; ○, Cremona; ×, Kanebian; △, Saran; ▲, Amilan.

acid. The treatment with coal tar was decidedly superior to any other treatments and its strength became about 45% of the initial stage after two months and thereafter rather rose and reached about 95% of the initial stage at the end of the experimental period. From this fact it may be considered that coal tar coated thread is not dried enough in the initial stages and frictional force between fibers diminishes and then fibers are apt to slip in loading. As coal tar dried gradually, fibers are stuck together by the pitch remained in coal tar and consequently the strength rises step by step.

B. Cremona

The cremona threads treated with palmitic acid, oleic acid, linseed oil and sardine oil diminished considerably in tensile strength respectively owing to the effect of exposure to the open air for two months and thereafter their strength were hardly diminished. The untreated thread (control) also followed almost the same as the course of the above treatment. The coal tar treated thread is superior to any other one and its strength was 63% of the initial strength after two months and 68% at the end of the six month.

C. Kanebian

Tensile strength of the Kanebian threads treated with oleic acid, linseed oil and sardine oil was diminished sharply owing to the effect of exposure to the open air and their strength decreased about 20% of the initial strength for two months. The threads treated with palmitic acid and the untreated thread followed nearly the same course. Strength of coal tar treated thread decreased 42% of the initial strength at the end of the second month but thereafter increased slightly, and consequently it may be considered that coal tar protects them from the effect of exposure to the open air.

D. Saran

The untreated thread and oleic acid, palmitic acid, linseed oil and sardine oil treated threads showed a tendency to weaken similarly and their strength decreased sharply and was about 35% of the initial strength after two months. Among them the strength of linseed and sardine oil treated thread rapidly decreased and was about 33~42% of the initial strength after one month. The strength of coal tar treated thread was 50% of the initial strength after two months but thereafter rather increased.

E. Amilan

This thread was affected remarkably by oleic acid, linseed oil and sardine oil and their strength was about 8~22% of the initial strength after two months and thereafter kept that state. The untreated thread and palmitic acid treated thread followed nearly the same course and their strength was about 13% of the initial strength after 4 months. The coal tar treated thread was better than the others and its strength was 60% of the initial strength after two months and thereafter rather increased.

II) On each substance treated

A. Coal tar

The coal tar treated threads diminished in strength owing to the exposure to the open air but the decrease of their strength was small compared with the other treated threads. Cotton thread was best of all the threads treated with

coal tar. The strength of all coal tar treated threads kept nearly above 50% of the initial strength during this experimental period.

B. Oleic acid

The oleic acid treated threads were remarkably weakened in July and Aug. by the exposure to the open air. In this case cotton thread was the worst and cremona was the best. Their strength was below 50% of the initial strength after 2 months.

C. Palmitic acid

The palmitic acid treated threads followed nearly the same course as the oleic acid treated threads did.

D. Linseed oil

The linseed oil treated threads were considerably weakened in Autum by exposure to the open air. Cremona was better than the others and the others except this followed nearly the same course. Cotton and amilan were inferior to the others.

E. Sardine oil

Sardine oil showed the more remarkable effect to threads than the other substances did when they were exposed to the open air, especially in Autumm. The strength of all sardine oil treated threads was under 40% of the initial strength after two months. Cotton and saran were bad, and kanebian and amilan followed the same course and cremona was superior to the others.

Summary

The principal results and conclusion the authors arrived at and the report in detail are here summarized.

1) Netting threads were treated with various oils and exposed to the open air.

2) The following oils were tested: Coal tar, oilic acid, palmitic acid, linseed oil and sardine oil.

3) The netting threads tested were cotton 20S/15, cremona 18S/18, kanebian 20S/15, saran 260D/18, and amilan 250D/12.

4) These threads were exposed to the open air at Kurihama (Kanagawa Pref.) from July, 1954 to Jan., 1955.

5) All netting threads showed some diminution of strength owing to the exposure to the open air during six months. The most loss rate of strength was sardine oil. It seemed that sardine oil had a remarkable effect to netting thread owing to the exposure.

Finally the authors wish to express their thanks to Messrs O. Suzuki, S. Mori, K. Kobayashi and K. Ozawa who help them in the experiment.

References

1) Fisheries Agency. 1950. The test reports on net and rope of synthetic fibers. pp. 1–105.
2) Okada, I. and others. 1952. On the efficacy of net preservatives. III. J. Tokyo Univ. Fish., 41 (1) 41–44.

Journal of the Tokyo University of Fisheries, Vol. 43, No. 2, 1957

POPULATION STUDIES ON THE JAPANESE IVORY SHELL, *BABYLONIA JAPONICA* (REEVE)

Tomokichi YOSHIHARA

(Received Jan. 16, 1957)

Table of contents

1. Introduction 207
2. Environments and oceanographic conditions 208
3. Methods of capture, measurement and tagging 209
4. Variability of catches per basket 211
5. Mechanism of trapping 217
6. Spawning, growth and survival 223
7. Natural increase rate 230
8. Estimation of population size 232
9. Summary 237
10. Appendix 237
11. Literatures 247

1. Introduction

The ivory shell, *Babylonia japonica* (REEVE) is a marine gastoropod commonly found in muddy sand. It is easily caught by a trap, the meat is edible, and the shell is used as the material of toys.

INO (1947, 1950) studied the life history and the method of propagation of this shell. KUBO and KONDO (1953) investigated the method of age-determination basing on the growth lines formed on the operculum. Since 1952, at Uchiura Bay in Chiba Prefecture the writer has been studying to obtain ecological information with reference to the population of this kind of shell in small bodies of water. The program involed the following elements: tagging experiments in which the shell caught were measured, weighed, tagged and released and their subsequent recoveries measured and weighed. By these means it was possible to obtain estimates of abundance. But the rate of growth, the tolal mortality rate, the net reproduction rate and the recruitment were estimated from other sources of data. Also the mechanism of fishing by the trap and the variability of catch by the trap were studied.

Most experimental informations come from the field research operated during each summer (May and July) in 1952–1956. A project of this sort requires the cooperation of a number of people. The writer wishes to acknowledge the valuable criticism provided by Prof. KUBO and Dr. INO, and it is a pleasure also to acknowledge the help throughout the investigations given by Messrs. U. UNO, T. MASUDA and T. FURUKAWA, the staff of the Kominato Marine Biological Laboratory and many students of our University.

Acknowledgment is also made to the Ministry of Education which made our present investigation the grant in aid for fundamental scientific research.

2. Environments and oceanographic conditions

Fig. 1 shows the map of Uchiura Bay and Fig. 2 shows the inlet within Uchiura Bay where the ivory shell has been found. The centre of Uchiura Bay lies approximately at Lat. 35°N. and Long. 140°E. Its long axis runs roughly north to south. The shoreline is rocky. The shape of the inlet is rectangular and the area comprises approximately 3×10^4 square meters. Its depth varies from 1 to 5 meters. The bottom consists of muddy sand, but there are rocky bottoms in various places. A fish market is situated on the south side of the inlet, and the waste of fish are thrown into everyday.

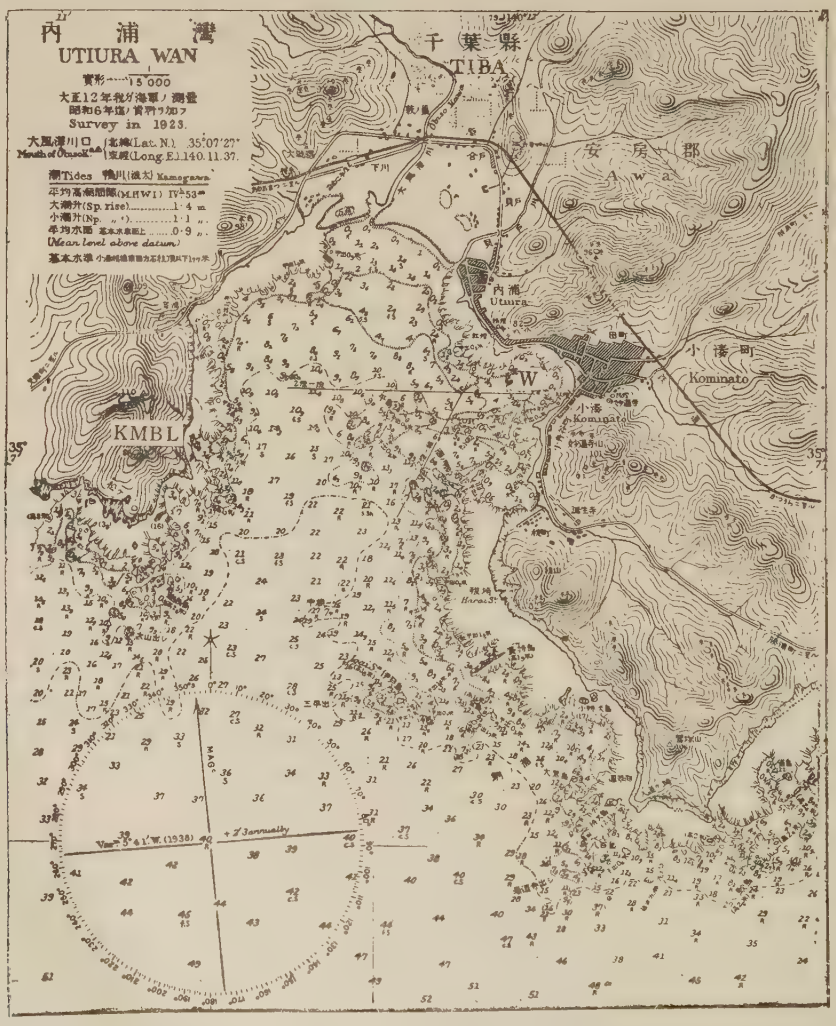


Fig. 1. Map of Uchiura Bay. W, inlet where samplings were done; KMBL, The Kominato Marine Biological Laboratory of the Tokyo University of Fisheries.

Based on the observations taken at the Kominato Marine Biological Laboratory we may show that the sea water temperature at Kominato increased about 1°C during the last two decades but no definite answer is now available as to whether the present warm period is drawing to a close or not. There are some effects of short term fluctuations of the oceanographic conditions on the abundance and distribution of fish, but a causal connection does not necessarily exist between the two, and no matter how strong the correlation, the proof of causation can be confirmed only by direct investigation of survival factors. Changes of the environmental conditions will affect the life history of organism in the sea, but the obvious effect on the survival rate or other biological characteristics of *B. japonica* is not obtained at the present stage of study.

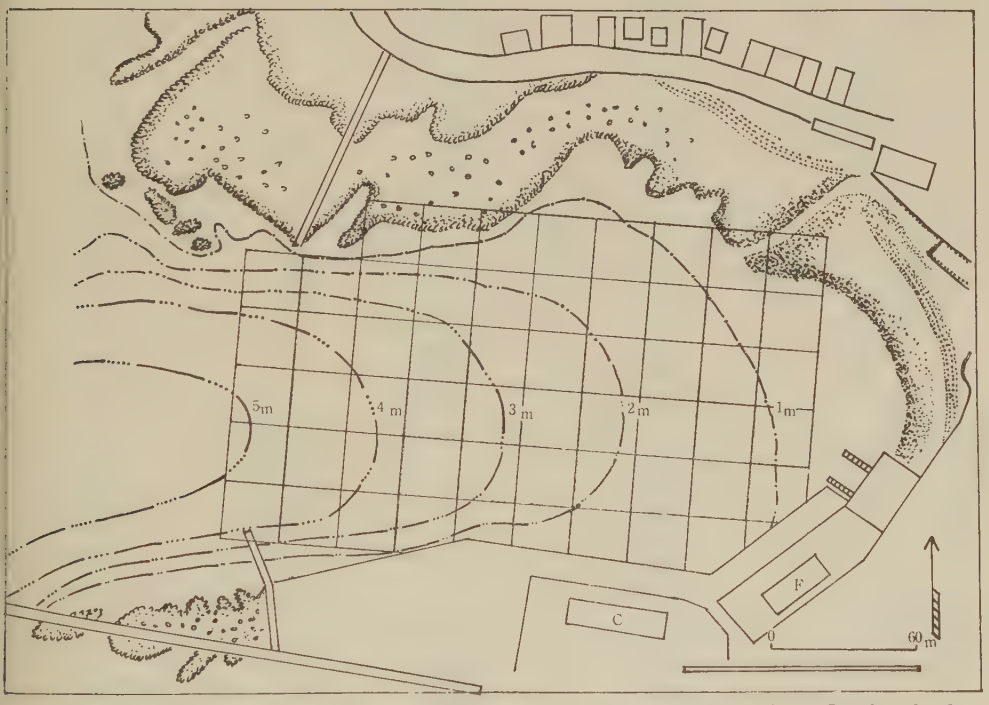


Fig. 2. The inlet within Uchiura Bay from where samples were taken. Quadrat in the inlet is used to show the density distribution of shell in section 8. F: fish market.

3. Methods of capture, measurement and tagging

Capture: This shell may be captured easily by a basket made of bamboo. A piece of fish flesh which is suspended within it by a skewer is used as bait (Plate. VII A and B). Ten or twenty baskets connected by the cotton rope are set down on the bed and are kept there about one hour. Then the shells which live within the sand come out and approach to the basket to eat the bait and at last fall in it (see Fig. 6). It was found that a shell may find the bait in the basket at a distance of five meters or more. One test was tried in the night and the same result was obtained.

The duration of sets is varied a little because of conditions and other factors, but most of the sets lasted one hour and not more than one hour. Thus, a unit of effort is available, which is the "catch-per-one-hour". When the baskets were pulled up, each shell was counted, tagged shell recovered, the number of the baskets used and kind of bait were recorded.

Table 1. Number of shell caught and efforts used to catch them in each year.

Date	Number of basket used × number of operation	Number of shell caught		
		Babylonia japonica (large)	*Babylonia japonica* (small)	*Nassrius* (*Noitha*) *livescens*
Oct. 4, 1951	20×1	278	—	307
5,	—	352	—	243
May 15, 1952	10×5	658	562	1103
16,	10×6	1254	271	561
July 9,	—	316	—	—
12,	—	286	—	—
May 9, 1953	18×1	340	108	79
10,	18×1	505	641	627
12,	18×1	395	224	181
June 11,	14×1	325	591	191
July 9,	18×1	166	84	123
10,	18×1	515	516	115
11,	18×1	248	246	101
May 11, 1954	20×1	455	94	79
12,	20×2	693	163	117
13,	20×1	183	31	88
July 21,	20×1	995	196	40
May 13, 1955	20×1	430	342	157
July 12,	20×1	310	92	61
13,	20×6	2156	427	125
14,	20×2	619	194	34
May 9, 1956	20×1	743	478	79
11,	20×2	191	250	9
12,	20×4	1771	878	80
July 11,	20×1	842	190	—*
12,	20×1	1030	229	—
13,	20×4	3274	836	—
14,	20×4	2840	855	—
26,	21×4	3422	761	—
27,	21×4	986	821	—
Total number of besket: 1052		26578	10091	4500

Number of operation: 59+3.
The length of large shell is 3 cm or more.
* Not counted

The records of catch are summarized in Table 1 and distribution of catch per basket is shown in Table 2. The Dog whelk, *Nassarius* (*Niotha*) *livescens* (PHILIPPI and other animals were caught at the same time but they were not measured.

Measurements: Length was read to the nearest millimeter, but, to facilitate analysis, in tabulation they were grouped 3 millimeter intervals; weight was taken to the gram and grouped 3 gram intervals. We could easily determine the sex

of each adult shell by the inspection of reproductive organs. Age was not determined.

Tagging: It is not the only difficulty to mark figures or signs on the surface of a shell by paint or lacquer, but to drill it is difficult for its surface is covered by epidermis and the shell is very hard. After several trials a satisfactory result was obtained by using the celluloid tag. The writer used a numbered small celluloid tag of different colour. It was attached on the shell by the fine copper wire. The tag had not caused any injurious effect on the growth of shell and kept about two years. Few of recovered shells had a hole showing a vestige of the tag.

This material is suitable for the small scale population study because:

i) it lives as a nearly closed population in the inlet where the investigation was done;

ii) it may be captured easily by a basket but fishermen do not catch them, which do not sell well there;

iii) the animal kept alive in the air about one day or more, so that we are to tag or to measure the length and the body weight of the shell without any injurious effect on it;

iv) the celluloid tag attached on the shell by the copper wire through a small hole drilled in shell, are not destroyed about two years;

v) it seems that the tag does not affect on the growth of shell;

vi) it is easy to find the recovered shell from the catch because the number of shell caught in one operation does not exceed a thousand.

4. Variability of catches per basket

The variability expected in replicate trapping, using a number of different baskets, has been examined. Some of the variability is due to true sampling variations, inevitable when discrete entities distributed in a plane and were attracted by the bait are sampled, and some is due to inadequacies of sampling technique such as the escapement of caught individuals by the lateral turning of a basket, or the setting of basket on the rock where no shell lives.

The baskets were immersed during same time interval in each operation to catch the shell but the distribution of shell on the bottom were different hence the uniform distribution could not be assumed.

The study of variation leads naturally to the concept of frequency distributions in which the frequency of the variable quantity under consideration takes each of its possible values. The detailed form of the distribution can be determined if a number of samples are available for the construction of frequency diagrams. In the present study as large number of sampling is done this has enabled frequency distributions to be set up as are shown in Table 2.

In samplings of our investigation the number of organisms is discrete, therefore the frequency distribution is essentially discontinuous. When there is a relatively small numer of organisms compared with elemental units of area, then with the organisms randomly distributed the sampling distribution is described by a Poisson distribution. One may write, $P(x=k)=m^k\, e^{-m}/k!$, where $P(x=k)$ donotes the probability that a sample will contain k organisms. Agreement

Table 2. Distribution of catch per basket

x denote the number of shell caught by a basket when the tail part (T) from 24 records of sampling extended from October 1951 to July 1954.

x	*B. japonica* (large)				*B. japonica* (small)				*N.* (*N.*) *livescens*				Grand Total
	T	H	N	Total	T	H	N	Total	T	H	N	Total	
0	16	12	11	39	19	25	24	68	25	19	23	67	174
1	6	6	3	15	9	11	21	41	15	16	20	51	106
2	2	—	5	7	9	7	14	30	8	10	10	28	65
3	3	4	5	12	11	8	8	27	3	7	9	19	58
4	2	3	5	10	4	5	5	14	3	11	5	19	43
5	3	5	4	12	2	5	7	14	7	4	11	22	48
6	2	1	8	11	5	3	2	10	4	4	7	15	36
7	2	3	8	13	1	5	6	12	5	2	2	9	34
8	5	3	8	16	4	2	5	11	2	4	6	12	39
9	1	1	2	4	1	3	1	5	4	5	1	10	19
10	3	1	4	8	2	5	1	8	3	4	9	16	32
11	—	2	1	3	3	1	2	6	3	2	5	10	19
12	3	5	2	10	3	3	—	6	1	3	—	4	20
13	4	2	3	9	1	1	1	3	3	—	2	5	17
14	1	2	1	4	2	1	—	3	—	1	—	1	8
15	—	2	4	6	—	4	—	4	1	3	1	5	12
16	2	—	4	6	3	1	1	5	1	2	4	7	15
17	3	2	4	9	2	—	—	2	3	2	1	6	17
18	—	4	5	9	1	—	1	2	—	1	1	2	13
19	3	3	3	9	1	—	—	1	—	1	1	2	12
20	1	3	2	6	1	1	—	2	—	1	1	1	9
21	—	2	2	4	1	1	1	3	—	—	—	—	7
22	4	2	2	8	2	3	—	5	3	2	1	6	19
23	1	3	7	11	1	—	1	2	—	—	1	1	14
24	2	—	3	5	—	1	—	1	—	—	1	1	7
25	—	1	4	5	2	—	1	3	1	—	—	1	9
26	1	—	2	3	3	—	1	4	—	—	—	—	7
27	3	3	3	9	—	—	—	—	—	—	—	—	9
28	1	2	—	3	—	—	—	—	—	—	1	1	4
29	—	1	—	1	—	2	—	2	—	—	—	—	3
30	1	1	5	7	—	—	1	1	—	—	2	2	10
31	1	—	1	2	—	—	—	—	—	—	1	1	3
32	1	3	1	5	—	1	—	1	—	—	—	—	6
33	2	2	4	8	—	—	1	1	1	—	—	1	10
34	—	2	1	3	—	1	—	1	—	—	—	—	4
35	—	1	—	1	—	1	—	1	—	—	2	2	4
36	1	1	1	3	—	—	—	—	—	—	1	1	4
37	—	2	1	3	—	1	1	2	1	—	—	1	6
38	1	1	—	2	1	1	—	2	—	—	1	1	5
39	—	1	—	1	—	—	—	—	—	—	1	1	2
40	—	—	3	3	1	—	—	1	—	—	—	—	4
41	—	1	—	1	—	1	—	1	1	—	—	1	3
42	1	—	—	1	—	—	—	—	—	—	—	—	1
43	1	—	1	2	—	—	—	—	—	—	—	—	2
44	—	—	—	—	1	—	—	1	—	1	—	1	2
45	2	—	—	2	—	—	—	—	—	—	—	—	2

classified by kinds of shell and the bait.

and head part (H) were used, N indicate unkown part of fish. Compiled

x	*B. japonica* (large)				*B. japonica* (small)				*N.* (*N.*) *livescens*				Grand Total
	T	H	N	Total	T	H	N	Total	T	H	N	Total	
46	1	—	—	1	—	1	—	1	—	—	—	—	2
47	2	—	—	2	—	1	—	1	—	—	—	—	3
48	—	—	2	2	—	—	—	—	—	2	—	2	4
49	1	1	—	2	—	—	—	—	—	—	—	—	2
50	1	—	—	1	—	—	—	—	—	1	—	1	2
51	—	1	—	1	—	—	—	—	—	1	—	1	2
52	3	1	1	5	—	—	—	—	1	—	—	1	6
53	—	—	1	1	—	—	—	—	—	—	1	1	2
54	—	1	—	1	—	1	—	1	1	—	—	1	3
55	—	—	—	—	1	—	—	1	—	—	—	—	1
56	—	—	1	1	—	—	—	—	—	1	—	1	2
57	—	—	—	—	1	1	—	2	—	—	—	—	2
58	—	—	—	1	1	—	—	1	—	—	—	—	2
59	—	—	1	1	—	—	—	—	—	—	—	—	1
60	—	1	1	2	—	—	—	—	—	—	1	1	3
61	—	1	1	2	—	—	—	—	—	—	—	—	2
62	1	2	—	3	—	—	—	—	—	—	—	—	3
63	—	—	—	—	—	—	—	—	—	—	—	—	0
64	—	—	—	—	—	—	—	—	—	—	1	1	1
65	1	—	—	1	—	1	—	1	—	—	—	—	2
66	—	1	—	1	—	—	—	—	1	—	—	1	2
67	—	—	—	—	—	1	—	1	—	—	—	—	1
68–71	—	—	—	—	—	—	—	—	—	—	—	—	0
72	1	—	1	2	1	—	—	1	—	—	—	—	3
73	—	1	—	1	1	—	—	1	—	—	—	—	2
74	—	—	—	—	—	—	—	—	—	—	—	—	0
75	—	1	—	1	—	—	—	—	—	—	—	—	1
78	1	1	—	2	—	—	—	—	—	—	—	—	2
77	1	1	—	2	—	—	—	—	—	—	—	—	2
78	—	—	—	—	—	—	—	—	—	—	—	—	0
79	—	—	—	—	—	1	—	1	—	—	—	—	1
80	—	1	—	1	—	—	—	—	—	—	—	—	1
81	—	—	—	—	—	—	—	—	—	—	—	—	0
82	—	1	—	1	—	—	—	—	—	—	—	—	1
83	—	—	—	—	—	1	—	1	—	—	—	—	1
84–86	—	—	—	—	—	—	—	—	—	—	—	—	0
87	1	—	—	1	—	—	—	—	—	—	—	—	1
88	—	1	—	1	—	—	—	—	—	—	—	—	1
89	1	—	—	1	—	—	—	—	—	—	—	—	1
90–101	—	—	—	—	—	—	—	—	—	—	—	—	0
102	1	—	—	1	—	—	—	—	—	—	—	—	1
103	—	—	—	—	—	—	—	—	—	—	—	—	0
104	—	—	—	—	—	—	—	—	—	—	1	1	1
163	—	—	—	—	—	—	—	—	—	1	—	1	1

between a theoretical and an exprerimentally determined distribution may be tested by means of the Chi squares test.

A supplementally test is to calculate the variance and its ratio to the mean. With a Poisson distribution this ratio, which has been termed the relative variance (CLAPHAM, 1936) or FISHER's coefficient of dispersion (BLACKMAN, 1942), is unity and the departure from unity is a measure of dispersion. In order to test this relationship the mean and variance of 24 records extended during 1951–1954 were examined. As is shown in Fig. 3, the Piosson distribution does not fit them.

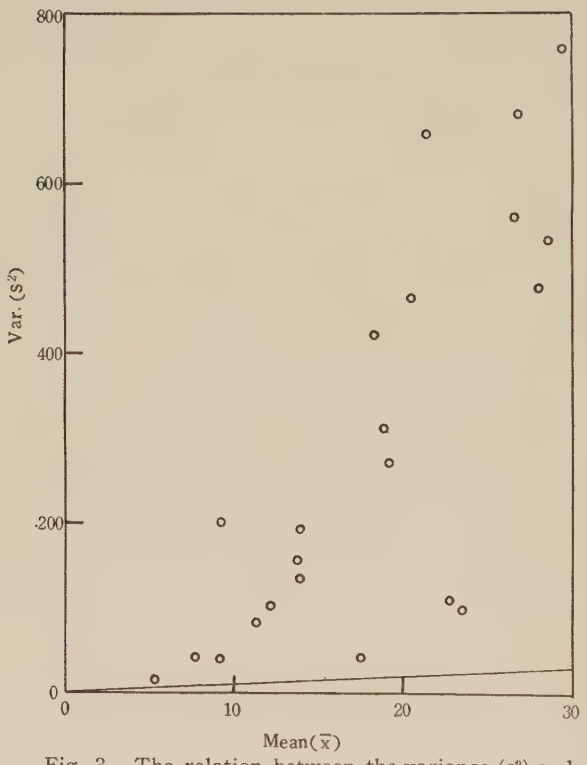


Fig. 3. The relation between the variance (s^2) and the mean ($\bar{x}$) of the distribution from series of catch data during 1951–1954.

It has been found that when a Poisson distribution does not fit their data there are often too many samples containing no individuals and too few containing one individual when compared with the appropriate Poisson series.

The general form of the frequency distributions, which were skewed and had variances greater than their means, indicated that they would require transformation before any analytical methods appropriate for normal distributions could be used.

The distribution of catches per basket is plotted on the semi-logarithmic paper as is shown in Fig. 4. It decreases linearly but for large values of the variable changes irregularly. Therefore for small values of the variable the distribution of the catch per basket may be represented by an exponential distribution.

On the other hand this type of distribution is described frequently by the negative binomial distribution. The frequencies are indicated by the expression

$$f(x)=q^{-k}\frac{(k+x-1)!}{x!\,(k-1)!}\left(\frac{p}{q}\right)^{x} \qquad (1)$$

where $p=m/k$, $q=1+p$, and m is the population mean of the distribution.

As a guide to an appropriate transformation, values of k may be useful. Estimate of k were obtained by using the equation:

$$k=\frac{\bar{x}^2}{s^2-\bar{x}} \qquad (2)$$

where $\bar{x}$ is the sample mean of the distribution and s^2 its sample variance.

The value of k for *B. japonica* (large) is 1.033 and is less than 2. Therefore a logrithmic transformation was indicated (Barnes, 1952).

The catch statistics on marine fisheries in Japan have been made by sampling methods since 1953, from which we have got a lot of records on catch per trip as the sample value. Based on the characteristics (such as the type of distribution) of catch per trip on various kind of unit fisheries the following results were obtained: (Yamamoto 1956)

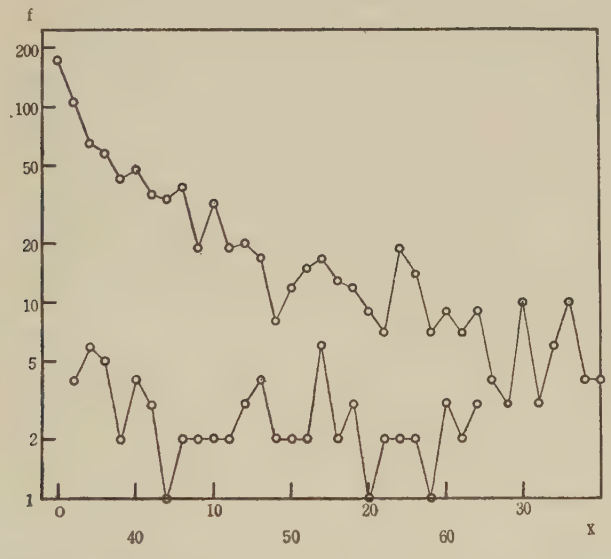


Fig. 4 Frequency distribution curve plotted on the semi-logarithmic paper showing the exponential distribution of the catch per basket. x: number of shell caught by a basket, f: number of basket containing x shells.

Types of distribution of catch per trip: These are approximately resembled to three types.

Type of distribution	Type of fishing	Coefficient of variance
1) normal distribution	trawling, longline fishery:	less than 0.5
1′) sub-logarithmic normal distribution		
2) logarithmic normal distribution	trawling, longline fishery:	0.5–1.0
2′) ultra-logarithmic normal distribution	fisheries using an echo-sounder or a fish gathering lamp, such as purse seine, dip net and angling:	
3) exponential distribution	drift gill net, set net, and beach seine, waiting for a fish schoal at the fishing ground:	greater than 1.0

It was accounted that the fisheries of type 1) are most stable fisheries and on the contrary, fisheries of type 3) are most unstable ones.

As the type of fishing in our investigation belongs to the type 3) the resulting frequency curve is the exponential type as might be expected. In fact the distribution of catch per basket is approximately represented by the exponential distribution curve (Fig. 4).

The effectiveness of logarithmic transformation in normalizing the original distribution is shown in Table 3. In right panel of Fig. 5 the transformed

Table 3. Normalized frequency distributions of the catch per basket by the logarithmic transformation

$\log x$	x	*B. japonica* (large)				*B. japonica* (small)				*Nassarius livescens*				Grand Total
		T	H	N	Total	T	H	N	Total	T	H	N	Total	
$-\infty$	0	16	12	11	39	19	25	24	68	25	19	23	67	174
0–	1	6	6	3	15	9	11	21	41	15	16	20	51	106
0.2—	2	2	—	5	7	9	7	14	30	8	10	10	28	65
0.4—	3	3	4	5	12	11	8	8	27	3	7	9	19	58
0.6—	4–6	7	8	17	33	11	13	14	38	14	19	23	56	127
0.8—	7–9	8	7	18	33	6	10	12	28	11	11	9	31	92
1.0—	10–15	11	14	15	40	11	15	4	30	11	13	17	41	108
1.2—	16–25	16	20	36	72	14	7	5	26	7	9	11	27	122
1.4—	26–39	12	20	19	51	4	7	4	15	2	—	9	11	76
1.6—	40–53	13	10	11	35	5	5	—	10	3	6	2	11	56
1.8—	54–99	6	8	—	14	2	4	—	6	—	—	1	1	21
2.0—	100–158	1	—	—	1	—	—	—	—	—	—	1	1	2
2.2—	159–	—	—	—	—	—	—	—	—	—	1	—	1	1
Total		85		97										
mean		1.192		1.227		(mean and variance were obtained by excluding the first row)								
variance		0.252		0.206										

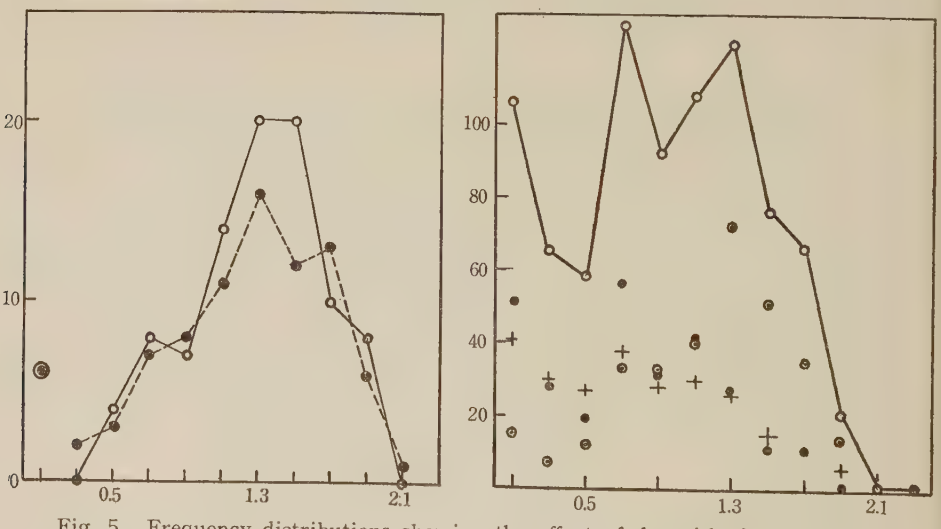


Fig. 5. Frequency distributions showing the effect of logarithmic transformation. Left panel: two frequency curves for each part of fish used as a bait for large shells, ●: tail part, ○: head part,. Right panel: four frequency curves, ○: grand total, ⊙: large shell, +: small shell, ●: *N. livecens*.

frequency distributions for the total of *B. japonica* and *N. livecens* and the grand total of these all frequencies are shown. Each frequency curve resemble to the normal distribution curve.

In the left panel of Fig. 5 two curves are shown by Table 3. Each of them corresponds to the result when a head and a tail of a bait fish (*Scomber japonicus*) were used. From these curves with the mean and the variance given in Table 3, it is evident that the effect of difference, between the parts of fish used as bait could not be found.

5. Mechanism of trapping

In order to capture the ivory shell we used a basket, within which a piece of fish was placed as bait. As the water in the inlet was turbid in summer we could not observe from the surface how rapidly the shell gathers around the basket at a time.

For each type of fishing the gear is set in water during an interval, and the number of caught fish increases according to the time elapsed after the gear had been set in water. But the velocity of the increase of catch during one operation can not be determined from various reasons. For example in the trawl fishing or longline fishery it will be impossible to observe the number of catch at each instance during the operation and the gear will be picked up before it becomes full of fish.

In our investigation, by observing at regular intervals, we may know by what rate the shell are gathering into the basket when the gear set down on the bed.

On each July in 1955 and 1956 we attemped to determine this rate by observing the movement of shells on the bottom around the gear using several divers. At first we made a lattice on the bottom by cotton ropes, one side of quadrat formed in the lattice was 5 meters and a basket was set down at the centre of a quatrat. About one minute later, when time was measured from this instant, shells, which hid in the sand, came out on the bottom and approached to the gear. The position of each shell was observed by the divers who went down in the sea at 1, 5, 10 and 20 minutes after the basket had been set down. The obervations were repeated seven times and the result is shown in Table 4.

In this Table 4, A, B, and C show the status of shell (see Fig. 6). In status A, shells are not appeared on the bottom yet, in status B, shells are creeping on the bottom. Most shell in status B approach to the basket, but some of them were leaving the basket. We treated these shells as those belonging to status B. The shell climbing the side of the basket was treated to belong to status B, too. After the shell had been caught it belongs to status C.

If the number of observations may be increased the more accurate result would be obtained, but under the conditions which the investigations were undertaken the method employed gave its best results, because, as we used divers, it was impossible to get continuous observations and 20 minutes after the basket had been set down the number of captured shell increased so that it could not

be counted exactly by divers. More frequent observations was impossible. After 30 minutes the basket was picked up and the number of shells was counted on the boat.

Now let us consider the rate of the change in the number of shells in each status. Assume that there are u, v, and w individuals in each status A,B, and C at time t. Then during the time interval $(t, t+\Delta t)$ number of individuals in

Table 4. Capture of *Babylonia japonica* by the basket. Showing the number of individuals in each status A, B, and C, figures in the first four rows are the sum of observed values of seven experiments, figures in the second four rows are shown as percentage, figures in last three rows are the theoretical values.

Time (minute)	t	1	5	10	20	30
Nmuber of individuals in status B	v	89	195	167	105	85
Number of individuals in status C	w	0	20	74	–	163
Sum of first and second row	$v+w$	89	215	241	–	248
Number of individuals remaining in status A	u	161	35	9	–	2
Percentage	v	35.6	78.0	66.8	42.0	34.0
	w	0	8.0	29.6	–	65.2
	$v+u$	35.6	86.0	96.4	–	99.2
	w	66.4	14.0	3.6	–	0.8
Theoretical values (percentage)	u	67.03	13.53	1.83	0.03	0.00
	v	32.1	73.6	67.2	42.0	25.5
	w	0.7	12.9	30.9	57.9	74.5

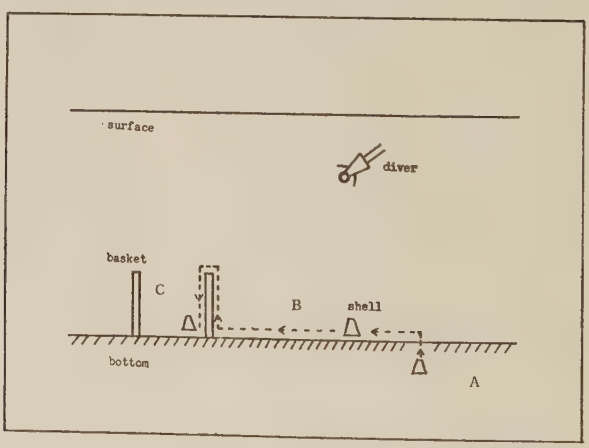


Fig. 6. Schematic diagram showing the movement of the shell on the bed.

status A will decrease because some of them transfer to status B. Let this number be Δu, then it will be proportional to the remaining member in status A, thus we get

$$\Delta u = -\lambda u \Delta t \qquad \text{(i)}$$

where λ is a proportional constant. During the same interval the number of

individuals in status B will be increased by the transfer from status A, but on the other hand it will decrease by the transfer to status C. If the decrease occures in proportion to the number of individuals in status B we get

$$\Delta v=\lambda u\Delta t-\mu v\Delta t \tag{ii}$$

where μ is a proportional constant. In status C the number of individuals will increase and it may be represented by the relation

$$\Delta w=\mu v\Delta t \tag{iii}$$

From these relations we have a simultaneous differential equation, when $\Delta t\to 0$,

$$\frac{du}{dt}=-\lambda u\,,\quad \frac{dv}{dt}=\lambda u-\mu v\,,\quad \frac{dw}{dt}=\mu v \tag{3}$$

with the initial conditions

$$u=N,\ v=0,\ w=0,\ \text{at } t=0 \tag{4}$$

where N is the total number of individuals existing in status A at $t=0$. The solution of these equations may be easily obtained, namely,

$$\begin{aligned} u(t)&=Ne^{-\lambda t}\\ v(t)&=N\frac{\lambda}{\lambda-\mu}(e^{-\mu t}-e^{-\lambda t})\\ w(t)&=N\frac{\lambda\mu}{\lambda-\mu}\left(\frac{1-e^{-\mu t}}{\mu}-\frac{1-e^{-\lambda t}}{\lambda}\right). \end{aligned} \tag{5}$$

At $t=0$ there is no individual in the status B hence $v(0)=0$, the value of $v(t)$ increase at first and at

$$t=\frac{1}{\lambda-\mu}\ln\frac{\lambda}{\mu} \tag{6}$$

it reaches maximum and then it decrease. After sufficient time all the individuals will be caught therefore $v(\infty)=0$. The v-curve has a unique mode.

It is easy to verify that $\int_0^\infty \frac{1}{N}v(t)dt=1$. Therefore we may treat the function

$$z=\frac{1}{N}v(t) \tag{7}$$

as if it were a probability density function and its mean and variance are

$$E(t)=\int_0^\infty t\,\frac{1}{N}v(t)dt=\frac{1}{\lambda}+\frac{1}{\mu} \tag{8}$$

$$\text{Var.}(t)=E(t^2)-E^2(t)=\frac{1}{\lambda^2}+\frac{1}{\lambda^2}\,. \tag{9}$$

From the observations by divers we may plot the value of $v(t)$ and $w(t)$ against time. If the number of individuals of status A is N at $t=0$ and there is no

recruitment from other domain the value of $v(t)+w(t)$ at $t\to\infty$ becomes N. From Table 6 it is evident that the value of $v(t)+w(t)$ increases rapidly and one hour later it will reach to about 250. This limiting value might be incorrect but we use it as N. (We use the total of seven observations for the convenience of calculations).

After determined the value of N we can get the values of $u(t)$ at each time by the relation $u(t)=N-v(t)-w(t)$. The value of $u(t)$ is given in Table 6, from which we can determine the value of λ by the equation (5) and it was found $\lambda\fallingdotseq 0.4$. The mode of $v(t)$ curve appears about $t\fallingdotseq 6$ minute. We know the values of λ and $t(\max)$ therefore the value of μ may be determined i.e. $\mu\fallingdotseq 0.05$.

All the values of N, λ, and μ determined by these methods are not accurate, but if there are many sufficient observations we can determine the values of parameters more exactly by the methods given in RUNNING (1917) or SANDEN (1914).

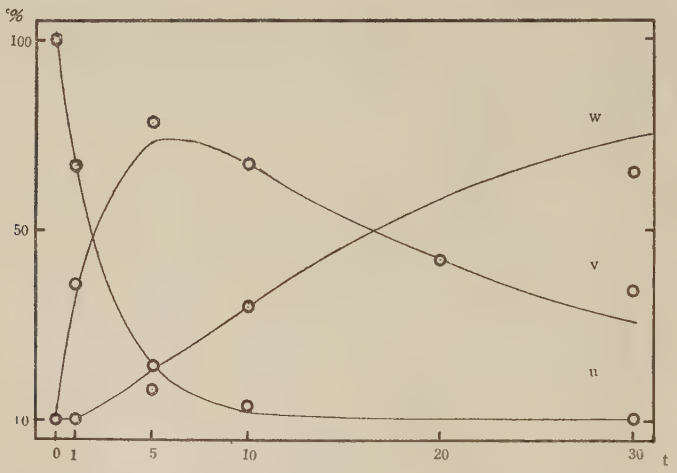


Fig. 7. The changes of position of individuals in each status A, B, and C.

u: number of individuals in A, i.e. number of shell which did not appears on the bottom by the time t,

v: number of individuals in B, i.e. number of shell which is creeping on the bottom,

w: number of individuals in C, i.e. number of shell caught

t : time elapsed since the basket is set down,

ordinate: percentage of number of individuals to initial number N in A.

Using the values thus obtained, theoretical values of u, v, and w were plotted in Fig. 7, and the agreement between the theoretically and the experimentally determined values was good.

5.1. Stochastic model on the mechanism of trapping

Figures in Table 6 is the total in seven experiments and in each experiment observing values showed considerable variations. Therefore it will be

interesting and necessary to treat this phenomenon by the stochastic method.

In stochastic models chance variation is introduced. The number of individuals which exist in status B are treated as random variables. Feller (1939) has discussed various stochastic processes in the study of the struggle for existence. For the general idea of stochstic processes in relation to the natural phenomena the reader is referred to the book of Feller (1950) and for the advanced study to the work of Kendall (1949) and others.

We shall denote by X the number of individuals existing in status B and X is treated as random variables assuming non-negative integral values. The probability that at time t there exist in status B exactly n organisms will be denoted by $P_n(t)$ which is a function of time t. We shall first derive the differential equations for the probability $P_n(t)$.

Now one of the simplest stochastic procesees is the Poisson process. This process postulates that whatever the number of changes during $(0, t)$, the probability that during $(t, t+\Delta t)$ a change occurs is $\lambda\Delta t+o(\Delta t)$, and the probability that more than one change occurs is of smaller order of magnitude than Δt. These conditions easily lead to a system of differential equations for $P_n(t)$.

In the Poisson process the probability of a change during $(t, t+\Delta t)$ is independent of the number of changes during $(0, t)$. The simplest generalization consists of dropping this assumption. We assume that, if n changes occur during $(0, t)$, the probability of a new change during $(t, t+\Delta t)$ equals $\lambda_n\Delta t$ plus terms of smaller order of magnitude of Δt. This type of process was first studied by Yule (1954) and Furry (1939) and is known as the pure birth process, but obviously it can not serve as a realistic model for changes in the size of populations whose members can die or similar problems. For these cases we can apply the birth and death process and our problem belongs to this type of process.

It is convenient to introduce here a more flexible terminology. Instead of saying that n changes occur during $(0, t)$, we shall say that the system is in state E_n. A new change then becomes a transition $E_n \rightarrow E_{n+1}$ in the pure birth process, but in the birth and death process the transition from the state E_n to next lower state E_{n-1} is permitted.

Then in order to exist n individuals in status B at time $(t+\Delta t)$ it must satisfy the following conditions. To calculate $P_n(t+\Delta t)$ we note that at time $t+\Delta t$ the system can be in state E_n only if one of the following conditions is satisfied:

i) at time t there are n individuals in status B (i.e. the system is in E_n) and during $(t, t+\Delta t)$ no change occurs;

ii) at time t there are $n-1$ individuals in status B (i.e. the system is in E_{n-1}) and one individual is added to the status B (i.e. a transition to E_n occurs);

iii) at time t the system is in E_{n+1} and a transition to E_n occurs;

iv) during $(t, t+\Delta t)$ two or more trasitons occur.

By assumption, the probability of the last situation tends to zero faster than Δt. The first three contigencies are mutually exclusive, so that their probability add. We get, therefore, the fundamental equations of birth and

death process. The coefficients λ_n and μ_n which appear in it can be arbitrarily prescibed and we assume that $\lambda_n=n\lambda$ and $\mu_n=n\mu$ which may be admitted from the treatment in the deterministic model. Then the differential equations become

$$\begin{aligned} P_0'(t)&=-N\lambda e^{-\lambda t}P_0(t)+\mu P_1(t) \\ P_n'(t)&=-(N\lambda e^{-\lambda t}+n\mu)P_n(t)+N\lambda e^{-\lambda t}P_{n-1}(t)+(n+1)\mu P_{n+1}(t) \\ P_N'(t)&=\ N\lambda e^{-\lambda t}P_{N-1}(t)-(N\lambda e^{-\lambda t}+N\mu)P_N(t)\ . \end{aligned} \tag{10}$$

At time zero the system is in state E_0, thus the initial conditions

$$P_0(0)=1\ ,\qquad P_n(0)=0\quad \text{for}\quad n\neq 0$$

hold.

The differential equations (10) uniquely determine the corresponding system of probabilities $P_n(t)$ but the explicit solution is rather complicated and could not be obtained by the elementary method. It is known that the solution may be obtained if we can solve the following partial differential equation

$$\frac{\partial \varphi}{\partial t}=(s-1)\left(N\lambda e^{-\lambda t}\varphi-\mu\frac{\partial \varphi}{\partial s}\right) \tag{11}$$

where

$$\varphi(s,\ t)=\sum_{n=-\infty}^{\infty}s^nP_n(t) \tag{12}$$

is a generating function.

Instead of solving this equation it is desirable to calculate the mean and variance of the distribution $P_n(t)$. Write for the mean

$$M(t)=\sum_{n=1}^{\infty}nP_n(t)\ .$$

Then differentiate with respect to t and using the relation (10) we get the relation

$$M'(t)=N\lambda e^{-\lambda t}-\mu M(t)\ .$$

This differential equation does not contain n so that we may solve it by the integration and get

$$M(t)=N\frac{\lambda}{\lambda-\mu}(e^{-\mu t}-e^{-\lambda t})\ . \tag{13}$$

This solution concide with the equation (5) (second formula) which is obtained by the deterministic method.

Similarly we write

$$M_2(t)=\sum_{n=1}^{\infty}n^2P_n(t)$$

then we have the differential equation

$$M_2'(t)+2\mu M_2(t)=(2N\lambda e^{-\lambda t}+\mu)M(t)+N\lambda e^{-\lambda t}$$

and solving this equation we get the solution

$$M_2(t)=N\frac{\lambda}{\lambda-\mu}(e^{-\mu t}-e^{-\lambda t})+\frac{(N\lambda)^2}{(\lambda-\mu)^2}(e^{-\mu t}-e^{-\lambda t})^2 .$$

Hence the variance of $P_n(t)$ is given by the expression

$$\text{Var.}P_n(t)=N\frac{\lambda}{\lambda-\mu}(e^{-\mu t}-e^{-\lambda t}) \qquad (14)$$

6. Spawning, growth and survival

The spawning season of *B. japonica* is from June to August. This shell is prolific, adult females of 6–7 cm in length being capable of producting about 10–60 eggs cases and each contains 27–50 eggs and average 42 eggs (Plate VII C and D). Thus in one spawning season at least about 500–2500 eggs are laid (Ino, 1950).

The size distribution of shell length obtained from 3000 measurements shows two modes at 2.5 cm and 5.6 cm. The first mode corresponds to the age I group and the second mode corresponds to age II or older age group respectively (Ino, 1950). However, it is impossible to separate the higher age groups from component groups by the length frequency distribution diagram. Hence we can not determine at what age the ivory shell matures.

This ambiguty may be cut out if the age of *B. japonica* can be determined. The method of age determination by the operculum was tried by Kubo and Kondo (1953). They distinguished the resting zones from r_1 to r_6. The result is shown in Table 5 with calculated shell length*.

Table 5. Calculated lengths and number of each age group of *Babylonia japonica* from Utiura Bay in 1952 (Kubo p. 203, Tab. 3, p. 204, Tab. 4)

Resting zone	r_1	r_2	r_3	r_4	r_5	r_6	r_7
Number of specimens	216	177	139	88	34	11	2
Length of operculum (cm.)	1.037	1.499	1.918	2.300	2.450	3.458	–
Shell length (cm.)	2.090	2.973	3.773	4.503	4.789	4.804	–

From the age composition of Table 5 the survival rate (ρ) of *B. japonica* may be obtained by Lea's formula as

$$\rho=\frac{177+139+88+34+11+2}{216+177+139+88+34+11}=\frac{451}{664}=0.679 .$$

The survival curve is shown in Fig. 8. The growth curve is shown in Fig. 8, too. The upper and lower bar indicate a 95 per cent confidence limits of shell

* They read all recoganizable zones without selection and further examined the strongly impressed resting zone. Later four resting zones were read out. From the tagging experiments it is known that some of the adult shell can survive more than three years after the tag has been attached. Therefore it is reasonable to believe that the shell can survive more than five years. Hence it is not legitimate to use the strongly impressed resting zone for the age determination.

length at each age. Average length corresponds to age V, VI, and VII appear to be too low.

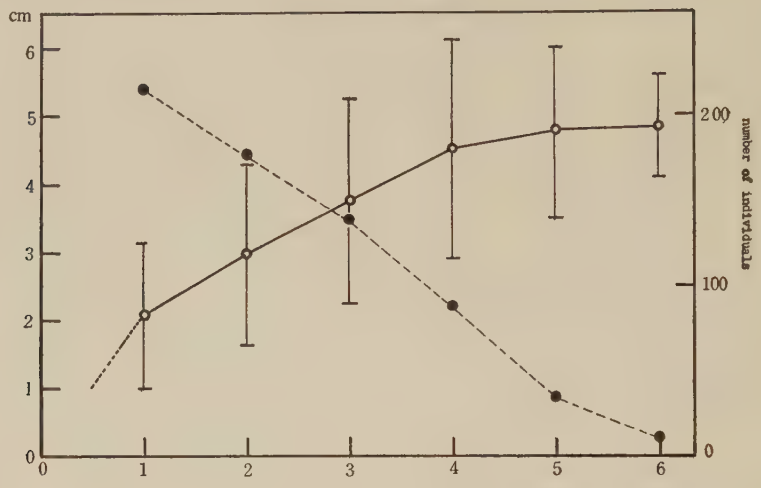


Fig. 8. Length-age relationship obtained from the age-determination by reading of the resting zone of operculum. Vertical line represents a 95 per cent probability range of size, dotted line indicates the survival curve. Abscissa: age.

6.1. Growth curve of *B. japonica*

As the shape of growth curve of this shell is sigmoid three kinds of growth curve were fitted They are

A: The negative exponential curve $l=L(1-e^{-ax})$ (15)

B: The logistic curve $l=\dfrac{L}{1+\exp(a-bx)}$ (16)

C: The Gompertz curve $l=L\exp(-\exp(a-bx))$ (17)

where $L=l(\infty)$ is the maximum shell length, a, b are constants and x is the age.

The WALFORD graph of length against that of previous year is shown as curve A in Fig. 9. Only the first four points fall on a straight line approximately parallel to the bisector of the axes. This fact indicates that the shell length increases at a constant rate namely the original growth curve is approximately a straight line. But at high age it converges to a point on a bisector.

For the curve B, which is used for both an individual growth curve and a population growth curve, the reciprocal of the shell length should be used for plotting points. Details of the method of constructing the diagram are given already (YOSHIHARA, 1951). In this example the transformed line is nearly straight and cuts the bisector at a point which does not coincide with the origin. Hence the logistic curve may be applied as the growth curve of *B. japonica*.

For the curve C, the equation may be transformed to the form

$$\log l=\log L-\exp(a-bx)\,. \qquad (18)$$

This is a curve of type A and the transformed line is nearly straight but it inflects slightly.

These results show that so far as we use the graphical methods the logistic curve is best fitted. When projected to the diagonal the theoretical maximum size of the shell obtained from the curve B corresponds to 5.0 cm approximately. The same value may be obtained from Fig. 8 and maximum shell length (upper confidence limit at age VI) is 6.1 cm in it. But the mode of shell length in each year, obtained from the length frequency distribution (Table 7), is larger than these values, thus it seems that the method of age determination by the operculum is not valid at high age although the reason of this fact is not evident.

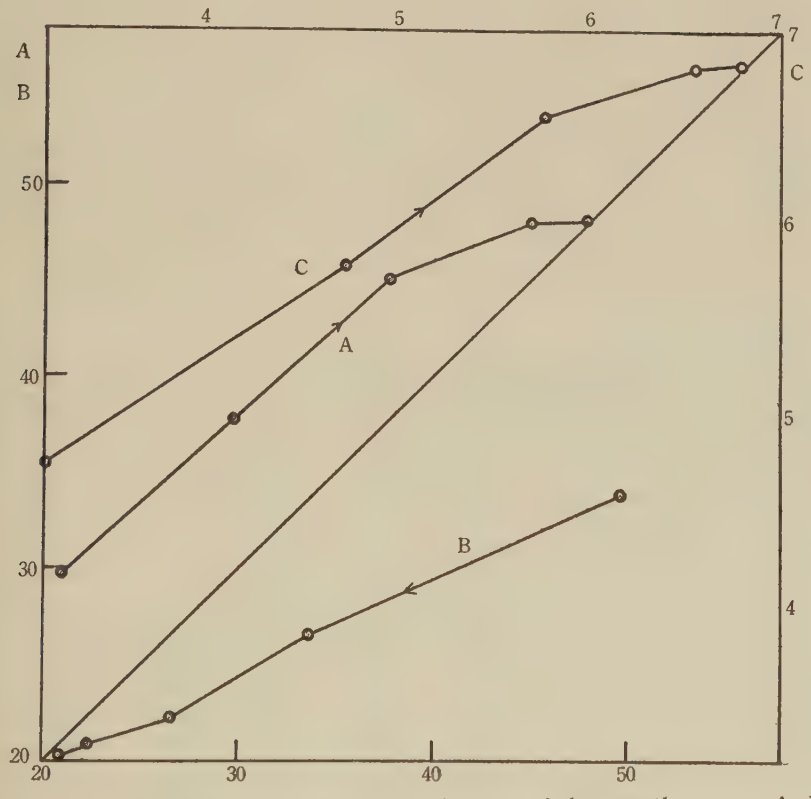


Fig. 9. Graphic method to determine the type of the growth curves A, B, and C given by equations (15)—(17).

From the tagging experiment we can confirm the growth of shell at the high age. Many tagging experiments have been done during the investigation but as this phenomenon had not been recognized previously, data to clarify this questionable point was not gathered. Length and weight frequency distributions by year and by sex are shown in Fig. 10 and Table 6. The mean values, modes and standard deviations are given in Table 7.

Table 6. Length and weight compositions by year and by sex

Shelll length (mm.)	Oct. 1951		July 1952		May 1953		July 1953		May 1954		July 1954	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
9–							29		–			
12–							129		3			
15–							137		43			
18–	3	–	–	–	–	–	273		37			
21–	8	–	–	–	–	–	74		31			
24–	12	–	–	–	–	–	54		11			
27–	16	1	–	–	1	–	18		2			
30–	13	1	–	–	3	3	10		2			
33–	8	3	–	–	2	7	9		3			
36–	8	3	–	1	4	9	4	2	–		1	–
39–	8	3	–	2	6	6	8	5	1	1	3	–
42–	12	6	–	1	6	7	10	8	1	1	15	–
45–	11	7	2	3	20	11	14	12	5	1	19	1
48–	18	10	7	8	29	19	35	15	17	7	42	9
51–	25	11	8	11	73	65	72	56	32	29	62	51
54–	27	35	8	28	114	74	110	67	75	76	117	74
57–	61	56	21	55	92	65	100	83	105	104	128	108
60–	56	51	27	44	77	53	83	72	74	115	116	103
63–	43	40	22	37	42	23	42	37	64	61	69	59
66–	19	13	4	16	11	10	15	19	15	26	28	24
69–	8	8	1	5	7	3	9	7	7	9	11	9
72–	–	–	–	–	–	–	–	1	1	1	4	1
75–	–	–	–	–	–	–	–	–	–	–	–	1
Total	356	248	100	211	487	355	1722		960		615	440

Body weight (gram)	May 1953		July 1953		May 1954		July 1954	
	♂	♀	♂	♀	♂	♀	♂	♀
3–	2	1	—	—	—	—	—	—
6–	3	11	—	—	—	—	—	—
9–	8	12	—	7	—	—	—	—
12–	7	10	3	6	3	1	2	—
15–	10	3	13	10	3	1	1	2
18–	23	15	12	11	2	3	5	10
21–	47	37	28	26	19	23	15	6
24–	81	54	26	50	18	34	9	16
27–	80	64	46	42	43	36	16	26
30–	79	43	48	53	55	70	12	11
33–	54	33	57	26	47	56	11	13
36–	34	28	42	33	69	60	7	11
39–	27	22	35	23	36	49	12	3
42–	12	11	18	9	42	48	3	4
45–	10	4	15	13	16	24	5	2
48–	10	7	2	8	14	14	2	1
51–	—	—	5	2	13	12	—	—
54–	—	—	—	3	7	3	—	—
57–	—	—	1	—	6	2	—	—
60–	—	—	—	—	3	2	—	—
63–	—	—	—	—	—	2	—	—
66–	—	—	—	—	1	1	—	—
69–								
Total	487	355	351	322	397	440	100	115

Table 7. Body length and body weight compositions. Means and standard deviations were obtained from the probability paper

Year			Length (mm)			Weight (gram)		
			Mode	Mean	S.D.	Mode	Mean	S.D.
Oct.	1951	♂	57–60					
		♀	57–60					
July	1952	♂	60–63	55	5.5			
		♀	57–60	57	5.5			
May	1953	♂	54–57	54	6.0	24–27	—	
		♀	54–57	53	6.0	27–29	27.0	
July	1953	♂	54–57	54	6.0	33–36	31.0	7.38
		♀	57–60	55	6.0	27–30	29.1	8.90
May	1954	♂	57–60	56	5.8	36–39	35.0	8.19
		♀	57–60	57	5.3	30–33	35.0	7.01
July	1954	♂	57–60	55	6.0	—	29.5	8.56
		♀	57–60	57	5.3	27–30	28.0	8.90

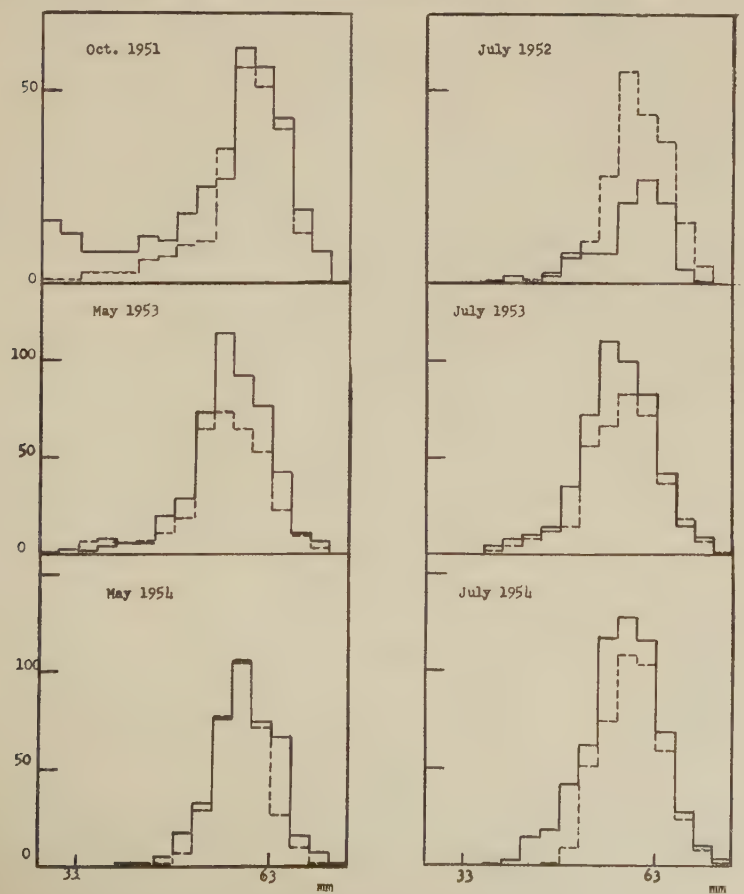


Fig. 10 (a). Frequency distribution of shell length by sex for each year. Dotted line indicate female.

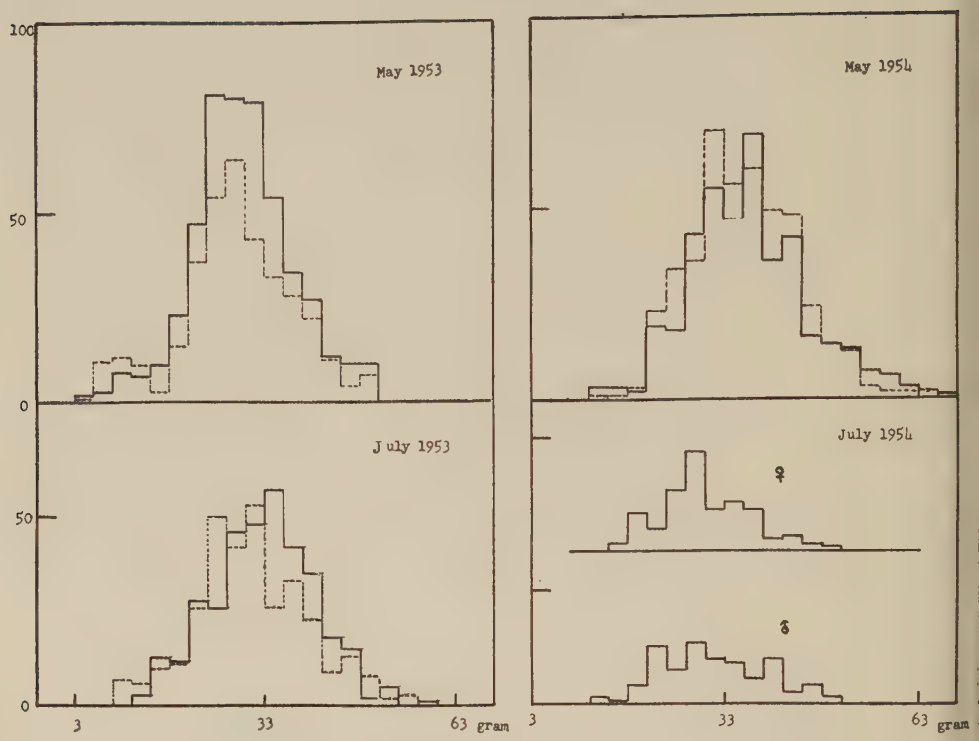


Fig 10 (b). Frequency distribution of shell weight by sex for each year. Dotted line indicate female.

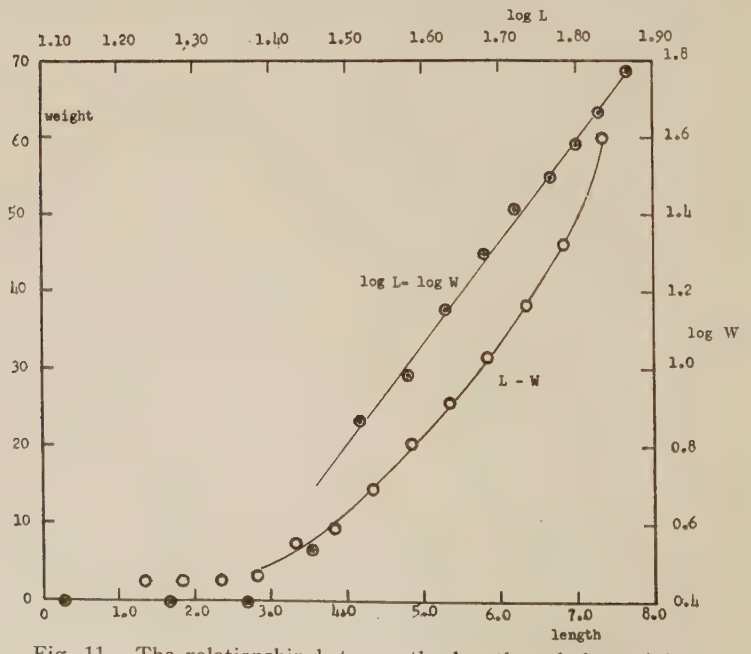


Fig. 11. The relationship between the length and the weight.

Table 8. Relationship between shell length and body weight based on 798 measurements made in July, 1956. Figures in first, second and third row show number of female, male and both sexes.

Length (mm)	Weight (gram) 0~	5~	10~	15~	20~	25~	30~	35~	40~	45~	50~	55~	60~	Total	Mean weight
11~	—													—	—
	1													1	2.50
	1													**1**	**2.50**
16~	1													1	2.50
	11													11	2.50
	12													**12**	**2.50**
21~	9													9	2.50
	15													15	2.50
	24													**24**	**2.50**
26~	29	2												31	2.82
	17	8												25	4.10
	46	10												**56**	**3.39**
31~	1	26												27	7.31
	—	10												10	7.50
	1	**36**												**37**	**7.36**
36~		17	8											25	9.10
		3	4											7	10.36
		20	**12**											**32**	**9.37**
41~			14	3	—									17	13.38
			10	9	1									20	15.25
			24	**12**	**1**									**37**	**14.39**
46~			2	11	19	1								33	20.38
			—	16	18	—								34	20.15
			2	**27**	**37**	**1**								**67**	**20.26**
51~				3	47	62	6	—						118	25.51
				2	48	56	12	1						119	25.90
				5	**95**	**118**	**18**	**1**						**237**	**25.71**
56~				1	1	36	45	11	—					94	30.90
				—	1	23	49	14	3					90	32.22
				1	**2**	**59**	**94**	**25**	**3**					**184**	**31.55**
61~					1	3	8	16	8	3	2			41	37.87
					—	1	8	22	16	2	—			49	38.52
					1	**4**	**16**	**38**	**24**	**5**	**2**			**90**	**38.22**
66~								1	5	2	1			9	44.17
								—	4	1	5			**10**	48.00
								1	**9**	**3**	**6**			**19**	**46.18**
71~												1	—	1	57.50
												—	1	1	62.50
												1	**1**	**2**	**60.00**
Total	40	45	24	18	68	102	59	28	13	5	3	1	0	406	
	44	21	14	27	68	80	69	37	23	3	5	0	1	392	
	84	**66**	**38**	**45**	**136**	**182**	**128**	**65**	**36**	**8**	**8**	**1**	**1**	**798**	
Mean length	26.75	34.67	41.75	48.56	51.82	55.00	58.17	61.21	64.92	65.00	64.67	73.00	—		
	23.45	31.81	41.57	46.70	51.60	54.56	57.71	60.84	63.22	64.67	68.00	—	73.00		
	25.02	**33.76**	**41.63**	**47.44**	**51.71**	**54.81**	**57.14**	**61.00**	**63.83**	**64.88**	**66.75**	**73.00**	**73.00**		

6.2. Length-weight relationship

The relationship between the shell length and the body weight has been determined. Fig. 11 shows this relationship for ivory shells from Uchiura Bay, and is based on a series of 798 measurements made in July, 1956.

The curve is fitted to the points, which was plotted by data given in Table 8. Both length and weight were recorded for many specimens taken during different years, but as the age had not been determined length and weight relationship for each age could not be obtained.

When the logarithm of the average weight is plotted against the logarithm of the mid-values of class interval of length in Table 8, the resulting curve for males and females approximates each other closely, both sexes are therefore combined and the points thus obtained tend to lie along a straight line.

The length-weight relationship may be expressed by the equation

$$W=0.2930\,L^{2\cdot8486} \tag{19}$$

where L is the length in centimeter and W the weight in gram.

7. Natural increase rate

The instrinsic rate of natural increase of animal populating may be determined from the growth curve of population or by calculating the net reproduction rate of natural increase. BIRCH (1953) estimated the natural increase rate for insect by both methods. He reared insects under carefully controlled conditions. From these data, life-tables and age-specific fecundity tables were constructed, enabling the natural increase rate to be calculated directly. Then he reared populations of the same insects at the same conditons. Logistic curves were found which adequately fitted as a growth curve of these populations. For the value of natural inarease rate calculated by these two entirely independent methods, the agreement between the two estimates was remarkably close. While for fish population in natural state it is not only difficult or impossible to observe the growth of population but also to calculate the net reproduction rate of natural increase, because there are very many eggs produced by female during a spawning seasen and most of larva die out at the early stages. Therefore we can not determine the natural increase rate neither by the direct way nor by the indirect one.

But if the poulation is in a stationary state and there are no death due to the commercial fishery the catchable population must be recruited by the new born indivduals. Through many investigations the age compositions of many kinds of fish population are known and we can get the survival rate from them. In almost all cases the apparent survival rate obtaned is constant or nearly constant throughout the life history except the early stage. Hence we may assume that the age specific rate of natural death is constant except the early stage.

Suppose that the number of individuals at the age x is n_x where $x=1, 2, 3,\cdots$ Then as we assume above $n_2=\rho n_1,\cdots n_x=\rho n_{x-1}$. Although the apparent survival rate obtained from the catch curve does not coincide with the ture survival rate of the population we shall usė it as if it were a true survival rate.

As the eggs spawned by adult female are abundant, if all of them grow on, the number of fish becomes very large. But under the natural conditions larva decreases very rapidly within a year by the predation and other causes of environmental or physiological conditions. Thus larva which hatched from the egg decreses to thousand or less within a year (see, for example, SETTE 1943).

If fish die exclusively by the physiological causes within a year after it hatch $n_1/\rho=n_0$ offsprings of age zero is need, to maintain the strength of fish population.

In the following we divide the produced eggs into two groups*, the first of which grow without death caused by the predation or other causes of environmental conditions. Therefore the survival rate of this group may be determined exclusively by the physiological factors. We shall call this group the offsprings of effective egg group**.

An alternative group of eggs consists of a vulnerable offsprings and die within a year not only by the causes due to the physiological factors but also by the predation or other causes of environmental conditions. Of course fish will be preyed at high age, too, but we can not estimate at which rate they are preyed. Therefore we shall use a rate which is the sum of natural decrease rate and the rate of predation i.e. the apparent natural death rate.

As the sex ratio of human and some of aquatic animal population (for example, fur seal population) under natural conditions is about 1:1, the net reproduction rate of these populations is usually calculated for female population only. The sex ratio of fish or shell population is not determined correctly. The obtained results varies considerably by season and by location. In some species the schoals are bisexual except during the spawning season (HOTTA and TAMURA, 1954). The sex ratio of *B. japonica* varies by season and the results, obtained from catch records in each year, varies considerably but as a whole the sex ratio seems to have the value 1:1. Thus we have assumed as if it were 1:1.

Suppose that female individuals of a population mature at age α, the effective offsprings produced at age x be m_x and in general from age α to age β female produce offsprings in each year. Usually in the fish population, the number of offsprings m_x increases linearly or exponentially as a function of age; thus it may be represented by the expression

$$m_x=m+kx \quad \text{or} \quad m_x=m\exp(kx), \quad \text{where } k \text{ is a constant} \qquad (20), (21)$$

because larger individual (fish or shell) produces more eggs, as could be expected. But as the first approximation, we can assume that m_x is constant throughout the reproductive age. This is true when the value of k is small.

There are few data available on the number of eggs actually laid under natural conditions. One of the method to assertain the spawned eggs is to examine the ovaries of the spent females which contain residual eggs but owing to the large variations of ovary eggs it is impossible to get exact data by this method (YOSHIHARA, 1952).

* This procedure is needed for the theoretical consideration but we can not designate them previously before they grow to some stage.

** This is equivalent to the effective litter size defined by COLE (1954).

As stated before most part of offsprings (produced) will die within a year. However, if we use the symbol m as an effective egg size we have the relation, when the population is in the stationary state,

$$ms^{\alpha}(1-s^{n})=1-s \tag{22}$$

where

m = the effective egg size
s = ρe^{-r}
r = the intrinsic rate of natural increase
ρ = the (apparent) survival rate
n = $\beta-\alpha+1$ (the length of spawning period)
α = the age at which the female initiates the spawning
β = the age at which the female adult terminates the spawning. Usually this age coincides with the highest age.

A formula which is equivalent to this formula may be obtained from the relation which gives the net reproduction rate of natural increase and used by the writer in the study of the regulation of fish population (YOSHIHARA, 1954). At the same time COLE (1954) had published a study based on the results obtained by THOMPSON (1931) and got similar relations assuming "the physiological survivorship". He had neglected the effects of the survival rate on the instrinsic rate of natural increase. Yet we can not neglect this factor because its influence on the fluctuation of fish population is very serious. In a similar manner described in this section we can discuss the effects of regulation or propagation on the abundance of fish population. Details of the results will be published elsewhere.

From above equation we can determine the number of effective egg size to maintain the strength of population. It should be noticed that the condition given by equation (22) is equivalent to $r>0$.

In *B. japonica* population we may assume that the sex ratio is 1:1, it mature at age 2 or 3, the surival rate is 0.679 and it survive to the age VI at least. Applying these values to the equation (22) we get, when $\alpha=2$,

$$m>(1-0.679)/0.679^{2}(1-0.679^{5})=0.813$$

in order to satisfy the condition $r>0$. Similarly we have $m=1.195$ provided $\alpha=3$. It is certain that the shell began spawning at the age of 2 or 3, hence the number of effective eggs must be $1\times2=2$ approximately because we had calculated the above value on female population only.

We may assume that the ivory shell lay 1000 eggs in a year at least. Thus when the population is in the stationary state $1-2/1000=99.8$ per cent of spawned eggs can not suvive to adult. From these considerations it is evident that if we protect indivuduals of early stage (of young shell population) the natural increase rate of the population will be greatly increased.

8. Estimation of population size

Abundance may be judged in several ways from data on hand. These include estimates of relative abundance from the special detailed catch statistics

for fishing, as well as calculations of actual abundance from the result of tagging experiments. In our investigation, as the samplings were done temporarily in each year there are weaknesses in data on catch per unit effort as bases for estimate of relative abundance*. Therefore the catch per unit of effort was not used.

The technique for estimating population size from detailed data on catch, or by sampling and removal, has been developed and described by DeLury (1947, 1051). Gerking (1952), Ketchen (1953) and Dickie (1955) compared estimate from this method with population estimate derived from the more familiar Schnable and Petersen tagging technique. As the ivory shell is not subjected to fishing and the catch by sampling is small compared with the population size we could not apply this method.

Dickie estimated the abundance of scallop population from the strip-census fishing technique. By the strip-fishing we may get the information which permit a calculation of the relative density taken in the strip fishing. Furthermore he made underwater photographic surveys to determine the density of the population of the areas sampled in the strip-fishing. But unfortunately at Uchiura Bay the conditions of the water does not permit the use of those useful methods. Therefore we had to apply a familiar tagging method and to make a map showing the relative abundance of the ivory shell in the inlet from which we had estimated the abundance of the population.

Table 9. Data of tagging and records of recapture

Date	Number of tagged shell (t)			Catch (n)													
				May 1953			July 1953		May 1954			July 1954	May 1955				
				9	10	12	10	11	11	12	13	21	13				
				340	505	395	515	248	455	693	183	995	430				
				n_1'	n_1''	n_1'''	n_2'	n_2''	n_3'	n_3''	n_3'''	n_4	n_5				
	Method of marking or tagging	Number		$n_1=1237$			$n_2=929$		$n_3=1331$			$n_4=995$	$n_5=430$				
				Recapture (s)													
May 16, 1652	Black paint	t_0'	100	t_0	400	3	—	—	—	—	—	2	3	4	1		
	Red paint	t_0''	200			—	1	—	—	—	—	—	—	—	—		
	White paint	t_0'''	100			1	—	3	2	1	2	—	—	1	—		
May 10, 1953	Yellow paint	t_1'	131	t_1	177					1	—	—	—	—	—		
June 11	Green cell.	t_1''	46							—	—	1	—	—	—		
July 10, 1953	Yellow cell.	t_2	328	t_2	328						2	4	2	6	1		
May 12, 1954	Pink cell.	t_3'	368	t_3	753									10	3		
	Green cell.	t_3''	385											7	2		
July 21, 1954	Green cell.	t_4	200	t_4	200										1		
May 13. 1955	Yellow cell.	t_5'	266	t_5	430												
	Pink cell.	t_5''	164														

[cell.=celluloid plate]

* In general, samples were taken twice per year (almost on the same month) so that they are not necessarily representative of the whole year.

There are several methods to give the abundance of population by the tagging experiment (YOSHIHARA, 1955). RICKER (1948, p. 39) stated assumptions which need to obtain the valid estimate by the tagging experiment. These are as follows:

i) that the survival rate of population is not affected by the attachment of a tag. The effet of this factor may be neglected in the ivory shell populaion.

ii) that the tagged shell does not lose their tag. When examining recaptured shell we found a shell which has only a hole but not the tag. These shell must lost the tag. Errors will be introduced by this cause in the estimation of abundance but as the number of these shell is small we had neglected the effect caused by the loss of the tag.

Apart from this factor when the experiment is extended over two or more years, the tagged shell will decrease about 30 percent during a year and two years after about 51 per cent by the natural death. Hence the abundance will be overestimated if we use uncorrected number of the recovery shell.

iii) that tagged shell mixed with untagged shell. This assumption is statisfied as the tagged shell was released all over the surface of the inlet.

iv) that all tags are recoganized and reported on recovery. This assumption is very important when catch size is very large. The tagged shells which contained in the catch are overlooked frequently but these were found by the re-examination, therefore it seems probable that almost all tagged shells that were recovered were found.

v) that the marked shells are as vulnerable to the sampling being carried on as are the untagged ones.

vi) that there is only a negligible amount of recruitment to the catchable population during the time the recoveries are being made: this assumption is essential to the estimate of population and is satisfied by the isolated and stationary population. The ivory shell population in the inlet seems to satisfy this condition because there are rocky beds outside the inlet and no shells live there.

At first the shell had marked by paint but this method did not gave good

Table 10 (i) Estimation of number of shell by Petersen's method and confidence interval of the abundance obtained from Chapman's table

Tagged number		catch n	$nt\times10^{-4}$	recapture s	$N\times10^{-4}$	$\underline{N}\times10^{-4}$	$\overline{N}\times10^{-4}$
t_0	400	n_1' 340	13.1	4	3.28	0.90	9.68
		n_1'' 505	20.2	1	20.2	1.65	39.40
		n_1''' 395	15.8	3	5.27	1.65	19.40
		n_1 1237	49.5	8	6.17	2.60	12.67
t_0+t_1	577	n_2' 515	29.7	2	14.85	2.28	84.0
		n_2'' 248	14.3	2	7.17	1.00	40.5
		n_2 299	53.5	4	13.36	3.69	39.4
$t_0+t_1+t_2$	905	n_3' 455	41.2	4	10.3	2.84	30.4
		n_3'' 693	62.7	7	8.96	3.52	19.4
		n_3''' 186	16.6	5	3.32	1.07	8.5
		n_3 1331	120.5	16	7.52	4.22	12.3
$t_0+\cdots+t_3$	1658	n_4 995	165.0	28	5.90	3.88	8.35
$t_0+\cdots+t_4$	1858	n_5 430	80.0	8	10.0	4.21	20.5

Table 10. (ii) Estimaton of number of shell by Schnabel's method

t		n		$n_4 \times 10^{-4}$	$\sum nt \times 10^{-4}$	s	$\sum s$	$(\sum nt/\sum s) \times 10^{-4}$
t_0	400	n_1	1237	49.5	49.5	8	8	6.19
t_1'	131	—	—	0	49.5	0	8	6.19
t_1'	46	n_2	929	4.27	53.8	4	12	4.48
t_2	328	n_3	1331	43.7	97.5	16	28	3.48
t_3	753	n_4	995	74.8	172.3	28	56	3.08
t_4	200	n_5	430	8.6	180.9	8	64	2.83

Table 10. (iii) Estimation of number of shell by Chapman's method (Model I)

t		May 1953	July 1953	May 1954	July 1954	May 1955
t_0	400	49.4	37.1	53.2	39.8	17.2
		$s_{11}=8$	$s_{12}=3$	$s_{13}=7$	$s_{14}=5$	$s_{15}=1$
t_1	177	$\frac{49.4}{8}=6.18$	21.9	23.6	17.6	7.6
			$s_{22}=1$	$s_{23}=1$	$s_{24}=0$	$s_{25}=0$
t_2	328		$\frac{108.4}{12}=9.02$	43.6	32.6	14.1
				$s_{33}=8$	$s_{34}=6$	$s_{35}=1$
t_3	753			$\frac{228.8}{28}=8.17$	75	32.4
					$s_{44}=17$	$s_{45}=5$
t_5	200		$\frac{\sum nt}{\sum s}=N$		$\frac{393.8}{56}=7.03$	8.6
						$s_{55}=1$
t_5	430					$\frac{472.3}{64}=7.37$
	n	1237	929	1331	995	430
		n_1	n_2	n_3	n_4	n_5

results because the shell surface does not easily dry, and figures or marks written on it frequently faded but some of marks remained four years. Small celluloid tag of different colour was used since 1953 and good results were obtained. But colour of tag had been faded after two years so that it was difficult to distinguish the colour of some tag.

Three methods were used to estimate the population size. The records of tagging experiment are shown in Table 9. If t shells are tagged and s are later recaptured among a catch of n shells then the tagging ratio in the population is t/N and in the sample s/n. Equating these two values we get the simplest formula for the estimation of population size i.e. PETERSEN's formula

$$N=nt/s\ . \tag{23}$$

However BAILEY (1951) has shown that a more nearly unbiased estimate is given by

$$N=(n+1)t/(s+1)\ . \tag{24}$$

If the successive tagging and recapture records may be utilized the abundance N will be given by SCHNABEL's formula (SCHNABEL 1938)

$$N=\sum nt/\sum s\ . \tag{25}$$

The estimates of abundance obtained form PETERSEN's and SCHNABEL's formula and CHAPMAN's method (CHAPMAN, 1954) are given in Table 10, from which we may conclude that there are about $3\sim10\times10^4$ adult shells in the inlet. On the other hand the area of inlet is about $130\times240\fallingdotseq3\times10^4$ square meters. Thus the density of adult shells in the inlet is from one to three individuals per square meter.

In summer of 1956 an investigation was undertaken to prepare the map of density distribution of shells to estimate the abundance of population.

A series of basket connected by the cotton rope was set at regular interval of 10 meters all over the bed of the inlet. The catch of each basket was examined and the results were ploted on a map. Each value was written in the quadrat of Fig. 1 and the mean of each quadrat was calculated. The result is shown in Table 11 with the number of sampling in each quadrat.

The basket was immersed just one hour and we may conclude that when the basket was immersed during one hour all the shell around it will be

Table 11. Density distribution of *B. japonica* in the inlet obtained from the repeated trapping. In each quadrat first row is sum of catch, second row is number of operation, third row is mean catch per one basket.

										Total
—	—	—	113	296	311	254	246	39	—	1279
—	—	—	2	3	7	4	5	1	—	—
—	—	—	66.5	98.7	44.4	63.5	49.2	39	—	—
83	208	249	403	415	203	249	502	65	10	2387
3	6	9	10	10	7	7	10	8	3	—
27.7	34.7	27.7	40.3	41.5	29.0	35.6	50.2	8.1	3.3	—
27	130	116	383	609	568	591	495	278	85	3282
2	4	6	11	10	10	11	11	6	2	—
13.5	32.5	19.3	34.8	60.9	56.8	53.7	45.0	46.3	42.5	—
49	409	344	277	579	458	374	475	352	303	3620
4	10	11	7	8	7	8	9	6	3	—
12.3	40.9	31.2	39.6	72.4	65.4	46.7	52.8	58.7	101.0	—
154	217	240	301	381	867	465	348	47	230	3250
4	7	7	5	7	12	8	8	4	2	—
38.5	31.0	34.3	60.2	54.4	72.2	58.1	43.5	11.8	115.0	—
—	61	122	310	222	181	29	16	52	—	993
—	5	3	6	3	3	3	2	1	—	—
—	12.2	40.7	51.7	74.0	60.3	9.7	8.0	52.0	—	—
Total 313	1025	1071	1807	2502	2588	1962	2082	833	628	14811
13	32	36	41	41	46	41	45	26	10	311
—	—	—	—	—	—	—	—	—	—	47.7

(Position of quadrat is shown in Fig. 1.)

caught. However, if one operates the trapping repeatedly after we had done some operations at the same position, almost equivalent catch may be expected. The reason of this phenomenon is not clear because at the first operation the shells which are vulnerable must be caught and residual individuals would be insusceptible to the existence of bait. One of plausible explanations of this phnomenon is that the shell which lives considerably remote from the basket may find the bait, but we can not give a reasonable explanation at present.

In Table 11 the mean catch per basket and the number of operation in each quadrat are shown with the total catch in each quadrat and the total of catch in all quadrat is 14811. In this table small shells are included but the number of them is not so large as is shown in Table 1.

The shell around the basket may find the bait and distance to it is about five meters. When a circle of radius is five meters, it comprises $5^2 \times 3.14 = 80$ square meters and if the radius of circle is 10 meters, it comprises about 320 square meters. One side of the quadrat is 24 meters hence its area is 576 square meters. Thus if the trappings were done a times in a quadrat and the mean catch per basket is b then the abundance of shells in this quadrat may be estimated from the formula

$$a \times b \times (576/80) \fallingdotseq 7ab \quad \text{or} \quad a \times b \times (576/320) \fallingdotseq 2ab\ .$$

Therefore the total size of shell poupulation in the inlet is about $7\,abc$ or $2\,abc$ where c is the number of quadrat.

In Table 11 total number of trapping is 311 and number of quadrat (c) is 54 hence $a = 331/54 = 6$ and the value of b is about $14811/311 = 50$. By means of these values we may calculate the relative abundance of shells in the inlet. When the radius of circle is five meters, it is

$$7 \times 6 \times 50 \times 54 \fallingdotseq 100{,}000$$

and if it is ten meters $2 \times 6 \times 50 \times 54 \fallingdotseq 30{,}000$. These values coincide approximately with the results obtained by the tagging experiment.

9. Summary

From data collected during 1951–1956 in the course of Uchiura Bay investigation some features of the ecology of the ivory shell, *Babylonia japonica* (Reeve) have been described.

The text contains the methods of reproduction and age determination described in previous publications which have been used to compute the survival rate, the natural increase rate, and to determine the type of growth curve.

From data collected more painstakingly the variability of catches per basket, the mechanism of the trapping, the abundance of population have been described.

10. Appendix

On the variability of catches per unit effort

Fisheries statistics are collected by various governmental agencies in several different ways, in greater or lesser detail, the method and form in each case depending on the ultimate use to which the information is put. A special

effort to collect detailed catch statistics has been made only in relatively recent years. The fisheries biologist collects catch statistics of fishery to determine what fluctuations in catch are taking place so that, if possible, he may determine the reason. He is concerned not only with total catch but also with the individual catches of each unit of gear, time and place of fishing, weather conditions, and any other factors which might directly or indirectly influence the success of fishing.

The author once examined the variability of catches of the Buri, *Seriola quiqueradita* by the trap net (YOSHIHARA, 1952). The catch data were obtained from the Kukiura fishing ground, Mie Pref. and included daily catches in weight over the period 1940–1949. The aspect of the Buri distribution in the space that bears on the problem of catch variability is the presence or absence of aggregation or schooling, for this affects the variability and determines the type of statistical distribution that fits the catches. There is evidence on the movement of school along the coast of Pacific side from south to north and when we plot the daily catches on the semi-logarithmic paper against time, peaks of catches appear. The writer had proved that we can get a logarithmic normal distribution on the catches of the Buri and later an extensive studies were done by YAMAMOTO (1956) and had been shown that under some conditions the distribution of catch per trip may be described by the exponential distribution. Based on this fact, the raw data on the catches of the Buri had been re-examined using the catches per day and found that it fit approximately to the exponential distribution. (see Fig. 12).

In the following it will be shown that when the original distribution may be described by the exponential distribution, an approximately normal distribution will be obtained by the logarithmic transformation.

Let x denote a random variable, λ a parameter, and the universe sampled has the frequency function $f(x)$, where $f(x)$ is greater than zero for $x \geqq 0$ and equal to zero elsewhere. Then (CRAMER, 1946, WILKS, 1947)

$$Pr(x<X<x+dx)=f(x)dx=\lambda e^{-\lambda x}dx \qquad (x \geqq 0) .$$

Applying the logarithmic transformation, $y=\log x$, the probability distribution function $f(x)$ becomes

$$Pr(y<\log X<y+dy)=g(y)dy=\lambda e^{y}e^{-\lambda \exp y}\,dy .$$

Now put $\lambda=e^{-\xi}$, $y-\xi=w$, then we have

$$g(w)=\exp(y-\xi-\exp(y-\xi))=\exp(w-e^{w}) . \qquad (26)$$

The mathematical expectation of an arbitrary function $\varphi(x)$, concerning the frequency function $\phi(x)$, denoted by application of the operator E, is

$$E(\varphi(x))=\int_{-\infty}^{\infty}\varphi(x)\phi(x)dx . \qquad (27)$$

In particular, if $\varphi(x)=x$, we have the mean

$$\tilde{x}=\int_{-\infty}^{\infty}x\phi(x)\,dx$$

and it will be assumed that $\tilde{x}$ is exist. When $\varphi(x)=e^{\theta x}$, where θ is a real variable,

(27) is called the moment generating function (m.g.f.).

Thus if we let $M(\theta)=E(e^{\theta x})$, we have

$$M(\theta)=E(e^{\theta x})=\int_{-\infty}^{\infty} e^{\theta x}\phi(x)dx. \tag{28}$$

If the k-th derivative of $M(\theta)$ with respect to θ exist, we have

$$[d^kM(\theta)/d\theta^k]_{\theta=0}=\nu_k \quad \text{where} \quad \nu_k=\int_{-\infty}^{\infty} x^k\phi(x)dx. \tag{29}$$

Thus the m.g.f. of x has the property that its k-th derivative at the origin gives the k-th moment of the distribution of x about the origin. In our case, as is given in (26), $\phi(w)=g(w)=\exp(w-e^w)$, thus putting $z=\lambda e^y$ we have

$$M(\theta)=\int_{-\infty}^{\infty} e^{\theta y}\, g(y)dy=\int_0^{\infty}\lambda^{-\theta}z^{\theta}e^{-z}dz=\lambda^{-\theta}\Gamma(\theta+1).$$

The logarithm of this expression gives

$$\log M(\theta)=-\theta(\gamma+\log\lambda)+\frac{1}{2!}S_2\theta^2-\frac{2!}{3!}S_3\theta^3\cdots$$

where

$\gamma=0.57721\ldots\ldots$(Euler's constant)

$S_2=\sum_1^{\infty} n^{-2}=\pi^2/6=1.64493$

$S_3=\sum_1^{\infty} n^{-3}=\ldots\ldots=1.20205$

$S_4=\sum_1^{\infty} n^{-4}=\pi^4/90=1.08232.$

Let the j-th moment about the origin and mean be ν_j, μ_i and the cumulant κ then we get

$$\kappa_1=\nu_1,\quad \kappa_2=\nu_2-\nu_1^2=\mu_2,\quad \kappa_3=\mu_3,\quad \kappa_4=\mu_4-3\mu_2^2$$

hence we have

mean	$=-\gamma-\log\lambda=\xi-\gamma$	
mode	$=-\log\lambda$	$=\xi$
variance	$=S_2$	$=1.64493\ldots.$
μ_3	$=-2S_3$	$=-2.40412\ldots.$
μ_4	$=6S_4+3S_2^2$	$=14.61136\ldots.$
skewness	$=\mu_3/\mu_2^{3/2}$	$=-1.13955\ldots.$
excess	$=\mu_4/\mu_2^2-3$	$=2.4005\ldots.$

Transfer the origin to mode and applying equation (26) we get Fig. 12 curve C, and curve B is the transformed distribution curve on the example of the catch per day by the trap net given in Tables 12-13. This curve agrees well with the transformed theoretical curve.

From the expression (26) we have for small value of w

$$\exp(w-e^w)=\exp(w-(1+w+w^2/2\cdots))\fallingdotseq e^{-1}\cdot e^{-w^2/2}.$$

From this expression it is evident that when the original distribution of catch per day or per unit effort may be represented by the exponential distribution function,

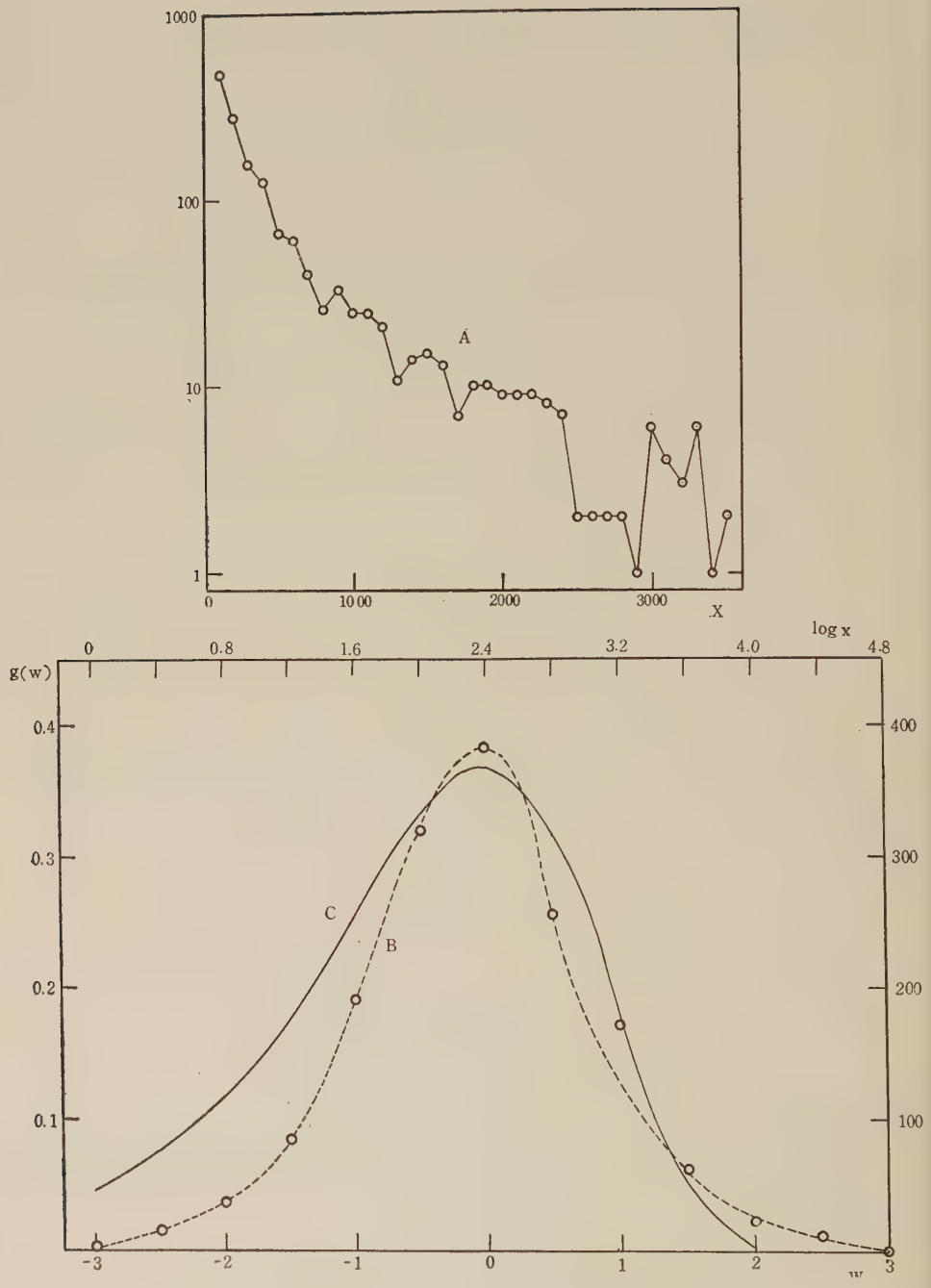


Fig. 12. Frequency distribution of the catch of the Buri by the trap net.
A: Exponential frequency curve based on Table 12.
B: Skew frequency curve based on Table 13.
C: Theoretical curve based on equation (26).

the transformed distribution curve approaches to the normal distribution curve near the mode.

We shall show further two examples which the original distribution is an exponential one.

Example 1. Distribution of catch by the tuna longline fishery

Some years ago the author published a paper on the distribution of catches by the tuna longline fishery, in which the horizontal distribution and the vertical distribution were treated. Since then the records of vertical distribution were frequently published in the reports of the investigation by the research vessel

Table 12. Catch of the Buri *Seriola quinquerdita* by the trap net at Kuki fishing ground, Mie Pref, during the years 1940-1949, where x denote the catch per day, unit in kan (1 kan=3.75 kg.)

	Year										
x	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	Total
0	13	15	18	19	21	28	13	12	12	11	162
1–	36	58	50	45	37	22	46	42	74	58	468
100–	38	38	22	19	14	18	23	22	40	33	276
200–	19	21	15	12	4	19	21	15	15	17	158
300–	14	8	17	7	7	17	17	16	9	15	127
400–	5	4	3	7	5	10	6	15	2	12	69
500–	2	4	5	6	6	5	12	6	6	9	61
600–	3	5	4	2	4	2	4	5	4	7	40
700–	4	3	1	4	2	4	3	2	3	0	26
800–	5	4	1	3	2	5	6	4	0	3	33
900–	4	2	3	1	2	2	2	4	0	5	25
1000–	3	1	1	2	4	5	2	3	1	3	25
1100–	3	1	4	4	3	2	1	3	0	0	21
1200–	1	1	2	1	3	1	1	0	0	1	11
1300–	2	2	2	0	2	1	1	3	0	1	14
1400–	1	0	3	0	0	5	2	2	1	1	15
1500–	3	0	1	0	5	1	2	1	0	0	13
1600–	1	0	1	1	1	0	0	2	1	0	7
1700–	1	0	1	0	0	3	3	1	1	0	10
1800–	0	2	3	1	2	2	0	0	0	0	10
1900–	1	0	0	0	0	3	1	1	2	1	9
2000–	—	1	—	1	2	2	1	1	1	—	9
2100–	—	—	1	—	3	5	—	—	—	—	9
2200–	2	—	1	3	—	1	—	—	—	1	8
2300–	1	1	1	—	1	—	—	3	—	—	7
2400–	1	—	—	—	—	1	—	—	—	—	2
2500–	—	—	1	—	—	—	1	—	—	—	2
2600–	—	—	—	—	1	—	—	1	—	—	2
2700–	1	—	—	—	1	—	—	—	—	—	2
2800–	—	—	—	—	—	—	—	1	—	—	1
2900–	—	1	—	1	—	3	—	—	1	—	6
3000–	4	—	2	6	3	2	1	3	3	1	25
4000–	3	—	—	2	4	4	1	4	—	—	18
5000–	3	1	5	4	2	3	3	4	1	0	26
10000–	2	0	6	3	1	4	3	3	2	0	24
Total*	163	158	156	145	120	152	163	167	167	168	1559

* In total, zero catch is excluded, and maximum catch is 42796 kan per day.

Table 13. Catch of the Buri, frequency distribution is obtained by the logarithmic transformation: x denote the catch by kan, $\log x$ the mid value of class interval

x	$\log x$	Year 1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	Total
0–1.6	0	0	0	1	1	0	0	0	1	0	0	3
1.6–	0.4	0	1	3	2	2	1	2	1	2	1	15
4.0–	0.8	1	3	4	6	6	0	7	1	7	2	37
10.0–	1.2	4	7	8	7	5	1	11	10	20	12	85
25.1–	1.6	19	25	22	17	11	9	17	16	31	23	190
63.1–	2.0	37	46	27	28	23	21	23	30	39	45	319
158.5–	2.4	45	43	39	31	15	43	50	38	40	39	383
398.1–	2.8	24	22	19	24	22	28	31	35	16	37	258
1000. –	3.2	21	9	20	13	25	33	13	22	7	8	171
2511. –	3.6	8	1	4	12	9	10	4	9	4	1	62
6309. –	4.0	3	1	5	2	1	2	4	4	1	0	23
15849. –	4.4	1	0	4	2	1	4	0	0	0	0	12
39811. –	4.8	0	0	0	0	0	0	1	0	0	0	1
Total		163	158	156	145	120	152	163	167	167	168	1559
Initiate from		Dec. 11	14	13	16	Jan. 15	Dec. 7	14	11	8	9	
Terminte on		May 29	30	31	24	31	31	Jan. 3	3	May 30	31	

of our country and general tendecies agreed with our result (YOSHIHARA, 1954).

In a recent study on the horizontal distribution of the catch by the tuna longline fishery the author has derived theoretically the horizontal distribution with an example based on the hypothetical values obtained from the various investigations. But it does not connected with the theme of this study so that it will be published elsewhere.

On the other hand the distribution of catch rate (i.e. catch per 100 hooks) by the tuna longline fishery was studied by Kuroki but the result is not published yet*. Therefore a frequency distribution table based on the records compiled by the Nankai Regional Fisheries Laboratory was prepared. As is shown in Table 14 it is clear that the distribution, in a yearly total, belongs to the exponential type distribution. We know that the number of operation during the summer (from May to September) is small and the catch is very poor while in the winter particularly from December to February we have very good catches.

Three frequency curves A, B, and C in Fig. 13 represent distributions of the total catch for the year 1949, sub-total catch of the good season (from December to February) and the poor season (from May to September), from which it is evident that the total frequency may be decomposed into two component ones. The first of them corresponds to the good season and the second the poor season.

According to the classification by YAMAOTO the type of distribution of the longline fishery belongs to the normal or log-normal distribution and this fishery

* Recently his paper has been published. (KUROKI, T. 1956. Theoretical estimation of albacore-shoals dimensions in accordance with the characteristic of fishing-rates, Mem. Fac. Fish. Kagoshima Univ. 5, 78–90). (added in proof).

Table 14, Distribution of the catch rate by the tuna-long line fishery for the year 1949. Constructed from the record gathered by the Nankai Regional Fisheries Research Laboratory.

Catch rate (x)	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
0	15	33	13	4	13	55	9	63	177	113	28	90	613
0–	33	69	46	14	2	5	6	38	53	134	94	95	590
1–	60	70	38	15	1	1	—	—	2	63	94	134	474
2–	68	71	31	12	—	—	—	—	—	33	70	111	396
3–	64	57	27	14	—	—	—	—	—	14	45	95	316
4–	61	34	27	8	—	—	—	—	—	11	30	74	241
5–	37	41	22	4	—	—	—	—	—	6	20	33	163
6–	32	22	22	8	—	—	—	—	—	1	12	18	115
7–	19	23	12	—	—	—	—	—	—	1	11	10	76
8–	19	14	9	—	—	—	—	—	—	1	7	3	53
9–	10	15	6	—	—	—	—	—	—	1	2	6	40
10–	14	11	7	—	—	—	—	—	—	—	—	2	34
11–	10	7	—	—	—	—	—	—	—	1	1	2	21
12–	11	2	2	—	—	—	—	—	—	1	3	1	20
13–	7	1	—	—	—	—	—	—	—	—	1	2	10
14–	4	2	—	—	—	—	—	—	—	—	1	—	7
15–	4	1	—	—	—	—	—	—	—	1	1	—	7
16–	2	—	—	—	—	—	—	—	—	—	—	1	3
17–	5	—	—	—	—	—	—	—	—	—	—	—	5
18–	1	1	—	—	—	—	—	—	—	—	—	1	3
19–	—	—	—	—	—	—	—	—	—	—	—	—	0
20–	—	1	—	—	—	—	—	—	—	—	—	—	1
21–	3	—	—	—	—	—	—	—	—	—	—	—	3
22–	1	1	—	—	—	—	—	—	—	—	—	—	2
23–	—	—	—	—	—	—	—	—	—	—	—	—	0
24–	1	—	—	—	—	—	—	—	—	—	—	—	1
25–	2	—	—	—	—	—	—	—	—	—	—	—	2
26–	1	—	—	—	—	—	—	—	—	—	—	—	1
32–	1	—	—	—	—	—	—	—	—	—	—	—	1
34–	1	—	—	—	—	—	—	—	—	—	—	—	1
35–	1	—	—	—	—	—	—	—	—	—	—	—	1
37–	—	1	—	—	—	—	—	—	—	—	—	—	1
45–	—	1	—	—	—	—	—	—	—	—	—	—	1

is stable one, however the fishery which has an exponential distribution of catch per trip belongs to the unstable one. His result was deduced from the distribution of catch per trip therefore it depends upon an average catch. We know that the distribution of mean form an arbitrary distribution approaches to the normal distribution, and the distribution of catch per trip of a vessel is a kind of mean of several catches extended during trip.

From these analysis we may conclude that although each catch per one operation of the tuna longline fishery has an exponential distribution in the poor season and a skew distribution in the good season we can get the normal or sub-log-normal distribution when we treat it as the catch per trip. Hence the long line fishery has a character of unstable fishery but it belongs to the stable fishery too.

Example 2. Distribution of catch in a creel census

In 1950 an investigation on the tagging experiments was undertaken by the Freshwater Fisheries Research Laboratory (1951) in the Nuru river, Gumma

Pref. This river is a branch of the Tone river and is isolated from the lower part of the river owing to the inflow of poisonous water containing sulphureous compounds from Shirane volcane. Thus no fish comes from the lower part of the river.

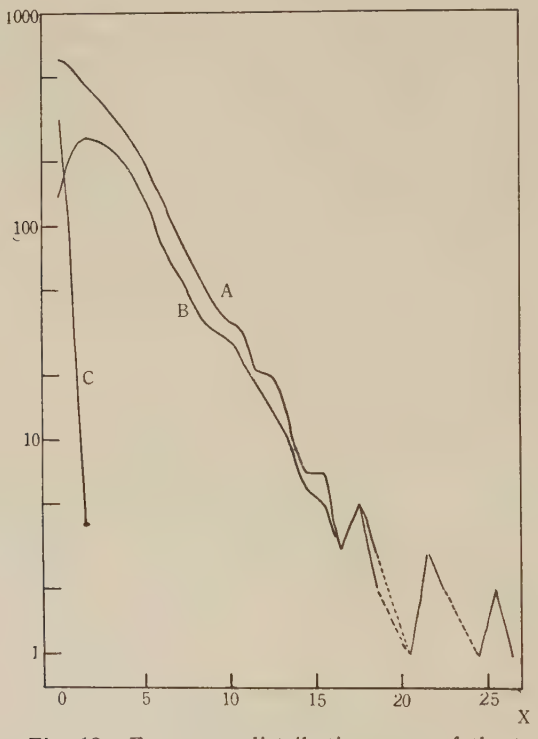


Fig. 13. Frequency distribution curve of the tuna longline fishery. x denote the catch rate.
A: Total catch for the year 1949.
B: Sub-total catch for the good season (December-February).
C: Sub-total catch for the poor season (May-September).

Twenty-eight thousand fishes (*Plecoglossus altivelis* (T. and S.)) were released from April to June and large part of them were marked by clipping the fin. A station was open in Ôto of Sakata-village. Two inspectors reside there and took records on catches of anglers, kinds of mark, methods of fishing, and locations of fishing ground along the river, etc. All anglers who enjoy the fishing as recreation and fishermen who live along the river have to pass there because there is no detour to lead them to the fishing ground except the way which passes there.

Fishing released from the prohibition on July 10. The records of catch on and after this day are summarized in Table 15. By means of the records given in this table we can draw the frequency distribution of catch by the angler and by the fisherman for each ten days interval and for whole period (Fig. 16). There

Table 15. Record of the number of catch during each ten days period. x denote the number of fish caught by angler (left) and fisherman (right) in one day. On the first day many fish were caught so its record is shown separately.

x	July 10		July 11 — 20		July 21 — 30		July 31 Aug. 9		Aug. 10 — 19		Aug. 20 — 29		Aug. 30 Sept. 8		Sept. 9 — 18		Total	Angler	Fisherman
	i		ii		iii		iv		v		vi		vii		viii				
1	3	—	6	4	3	1	1	6	4	9	5	8	1	3	1	4	59	24	35
2	2	3	—	4	3	1	6	7	5	5	3	2	2	1	—	2	46	21	25
3	4	4	6	12	4	7	3	15	8	8	3	3	4	1	1	1	84	33	51
4	4	3	6	10	3	10	3	19	8	8	4	1	3	1	—	—	83	31	52
5	3	2	4	5	1	7	4	11	7	8	1	2	—	—	—	—	55	20	35
6	5	2	9	6	14	6	4	14	5	3	3	1	—	—	—	—	72	40	32
7	7	1	11	3	4	4	9	12	5	7	2	—	—	—	—	—	65	38	27
8	7	5	11	6	5	11	7	10	1	2	1	—	—	—	—	—	66	32	34
9	5	1	2	2	3	7	4	4	5	1	1	—	—	—	—	—	35	20	15
10	9	2	4	4	3	11	3	8	7	5	1	—	—	1	—	—	58	27	31
11	11	2	5	4	4	13	8	3	4	2	—	—	—	—	—	—	56	32	24
12	8	3	6	1	3	6	5	8	—	1	—	—	—	—	—	—	41	22	19
13	6	2	2	—	3	8	2	1	3	1	—	—	—	—	—	—	28	16	12
14	4	—	4	4	2	1	6	1	1	—	1	—	—	—	—	—	24	18	6
15	1	—	4	5	2	9	4	2	3	—	—	—	—	—	—	—	30	14	16
16	2	—	3	—	2	3	5	4	1	—	1	—	—	—	—	—	21	14	7
17	3	1	2	3	2	4	5	2	1	1	—	—	—	—	—	—	24	13	11
18	4	1	—	2	2	7	—	1	—	—	1	—	—	—	—	—	18	7	11
19	3	—	1	1	1	2	2	3	2	—	—	—	—	—	—	—	15	9	6
20	3	—	1	3	1	5	2	—	—	—	—	—	—	—	—	—	15	7	8
21	2	—	—	2	—	4	3	—	1	—	—	—	—	—	—	—	12	6	6
22	3	1	3	—	—	1	6	—	—	—	—	—	—	—	—	—	14	12	2
23	3	—	1	1	1	7	2	—	—	—	—	—	—	—	—	—	15	7	8
24	2	—	1	—	1	1	4	—	—	—	—	—	—	—	—	—	9	8	1
25	1	1	2	2	2	1	5	1	—	—	—	—	—	—	—	—	15	10	5
26	2	—	1	1	2	—	5	—	1	—	—	—	—	—	—	—	12	11	1
27	1	—	1	—	—	—	4	—	2	—	1	—	—	—	—	—	9	9	0
28	1	—	1	—	—	—	2	1	1	—	—	—	—	—	—	—	6	5	1
29	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	3	3	0
30	2	—	1	—	—	—	3	1	—	—	—	—	—	—	—	—	7	6	1
31	1	—	—	1	—	2	—	—	—	—	—	—	—	—	—	—	4	1	3
32	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	1	0
33	1	—	1	—	—	1	—	—	1	—	—	—	—	—	—	—	4	3	1
34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0
35	—	—	—	1	1	1	1	—	—	—	—	—	—	—	—	—	4	2	2
36	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2	0
37	1	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	3	2	1
38	—	—	—	—	1	1	1	—	—	—	—	—	—	—	—	—	3	2	1
39	1	—	—	—	1	—	2	—	—	—	—	—	—	—	—	—	4	4	0
40	1	—	—	2	1	—	2	—	1	—	—	—	—	—	—	—	7	5	2
41	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	2	2	0
42	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	2	1	1
43	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	1	0
44	1	—	1	—	1	—	1	—	1	—	—	—	—	—	—	—	5	5	0
45	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	2	2	0
46	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	2	2	0
47	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0
48	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0
49	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	1	0
50	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	1	0
Total	123	35	102	89	80	142	130	134	78	61	28	17	10	7	2	7	1046	553	493

(Compiled from unpublished original records.)

are 1046 records which provided by anglers and fishermen and they contain almost equal number of records from anglers and fishermen but the record on zero catch could not obtained.

By the logarithmic transformation we can get a frequency distribution in Table 16. In both Tables 15 and 16 the zero catch is not recorded thus omitting it the means and variances were calculated as shown below:

	Mean		Variance	
	Angler	Fisherman	Angler	Fisherman
Table 15	12.8644	9.0101	108.5928	54.4522
Table 16	0.9854	0.8380	0.1495	0.1498

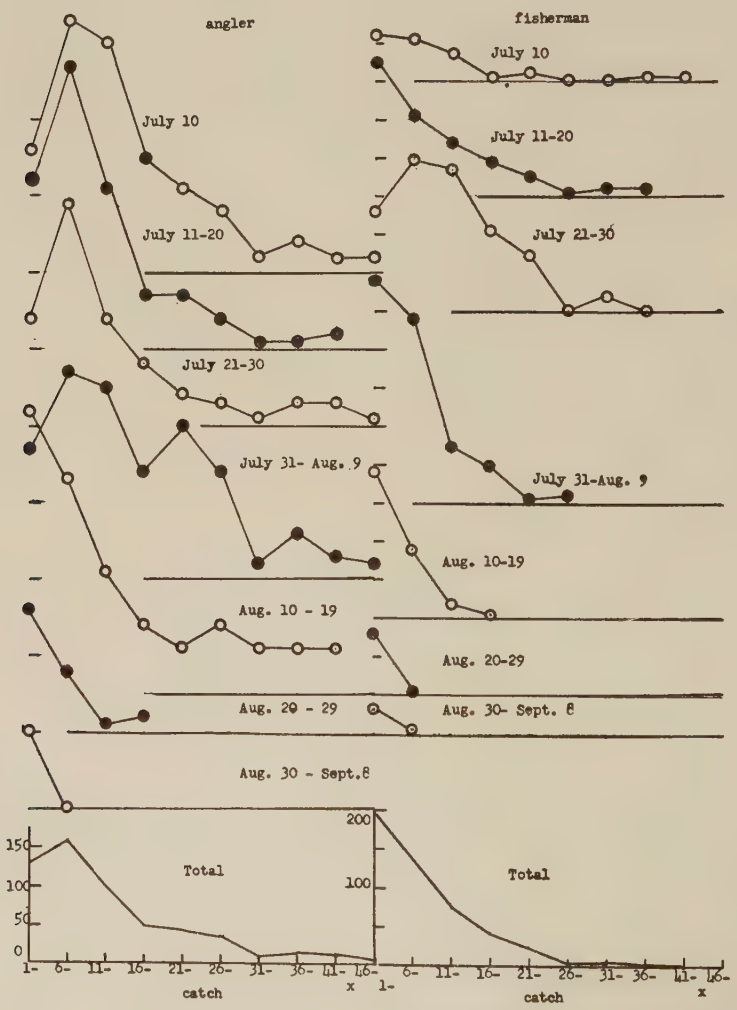


Fig. 14. Frequency diagrams of catch by anglers and fishermen for each ten days interval and the whole period, left panel shows the distribution for anglers and right panel for fishermen.

From this result and the frequency diagrams in Fig. 15 it is evident that there is no significant difference between the means of catch by anglers and fishermen.

Table 16. The transformed frequency distribution of the catch in Table 15. $X=\log x$ denote the mid-value of the interval.

x	1	2	3	4–6	7–9	10–15	16–25	26–39	40	Total
X	0.1	0.3	0.5	0.7	0.9	1.1	1.3	1.5	1.7	
f_1	24	21	33	91	90	129	93	51	21	553 (Angler)
f_2	35	25	51	119	76	108	65	11	3	493 (Fisherman)

In the frequency diagrams for each ten days interval shown in Fig. 14 the shapes of it in later season approach to the exponential type distribution. The skew frequency distribution may be attained from the records by good anglers in the good fishing ground or season, on the contrary an exponential frequency distribution will be yield when poor fishermen fished on the bad fishing ground or season.

Similar results were obtained by Calhoun (1950) in the investigation undertaken in California on the freshwater fishing. His results show an exponential type frequency diagram. The curve for salmon decrease most rapidly and that of trout most slowly. Hence it seems that the degree of difficulties in fishing for salmon is highest and that of trout is lowest, while that of bass is in the middle rank.

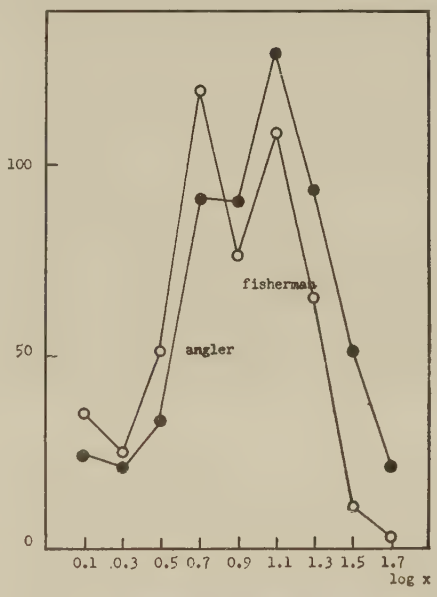


Fig. 15. Frequency diagrams of catch by anglers and fishermen based on the logarithmically transformed frequency distribution.

Literatures

Bailey, N. T. J. 1951: On estimating the size of mobile populations from recapture data, Biom. **38**, 293–306.

Barnes, H. 1952: The use of transformations in marine biological statistics, J. du Conseil Int. Expl. Mer. **18**, 61–71.

Birch, L. C. 1953: Experimental background to the study of the distribution and abundance of insect, I and II., Ecology, **34**, 698–711, 712–726.

Calhoun, A. J. 1951: California state-wide angling catch estimate for 1949, Calif. Fish and Game, **37**, 69–75.

Chapman, D. C. 1948: A mathematical study of confidence limits of salmon populations calculated from sample tag ratios, in Problems in enumeration of populations of spawning sockeye salmon, Part. 2, Bull. Intern. Pacific Salmon Fish. Comm. II, 67 85.

———— 1954: The estimation of biological populations, Ann. Match. Stat. **25**, 1–15.

Cole, L. C. 1954: The population consequences of life history phenomena, Q. Rev. Biol. **29**, 103–137.

CRAMER, H. 1946: Mathematical methods of statistics, pp. 185–7.

DE LURY, D, B. 1947: On the estimation of biological populations, Biometrics, **3**, 145–167.

DICKIE, L. M. 1955: Fluctuations in abundance of the giant scallop, *Placopected magellanicus* (GMELIN), in the Digby area of the Bay of Fundy, J. Fish. Res. Bd.Canada, **12**, 797–857.

FELLER, W. 1950: An introduction to probability theory and its applications, I. pp. 418.

Freshwater Fishery Research Laboratory. 1951: Investigations on the release of Ayu in a river, Naisuimen Gyogyo Shiryo, No. 16. (in Japanese).

GERKING, S. D. 1952: Vital statistics of the fish population of Gordy Lake, Indiana, Tr. Amer. Fish. Soc. **82**, 48–67.

HOTTA, H. and TAMURA, T. 1954: An ecological note on the Shirauo, *Salangichthys microdon* Bleeker, Bull. Fac. Fish. Hokkaido Univ. **5**, 41–46.

INO, T. 1947: Report on progress of a test for propagation of the Japanese ivory shell, *Babylonia japonica* (REEVE), A phamplet issued from the Fisheries Experimental Station of Chiba Pref. pp. 1–10. (in Japanese)

——— 1950: Life history of Japanese shell, *Babylonia japonica* (REEVE), and its propagation method. Studies on aquatic animals, No. 1. pp. 11–21. (in Japanese)

KENDALL, D. G. 1949: Stochastic processes and population growth, J. Roy. Stat. Soc. (B) **11**, 230–282.

KETCHEN, K. S. 1953: The use of catch-effort and tagging data in estimation a flatfish population. J. Fish. Res. Bd. Canada. **10**, 459–485.

KUBO, I. and KONDO, K. 1953: Age-determination of the *Babylonia japonica* (REEVE), an edible marine Gastropod, basing on the operculum, J. Tokyo Univ. Fisheries, **39**, 199–207.

Nankai Regional Fisheries Laboratory, 1951: Supplementary report No. 1, pp. 194.

RICKER, W. E. 1948: Method of estimating vital statistics of fish population, Indiana Univ. Publ. Sci. Series No. 15, pp. 101.

RUNNING, T. R. 1917: Graphical formulas, pp. 142.

SANDEN, H. von, 1914: Praktische Analysis.

SCHNABLE, Z. E. 1938: The estimation of the total fish population in a lake, Amer. Math. Monthly, **45**, 348–352.

SETTE, O. E. 1943: Biology of the Atlantic Mackerel (*Scomber scombrus*) of North America, Part. 1: Early life history, including the growth, drift, and mortality of the egg and larval population, Fishery Bull. **38**, 149–237.

WILKS, S. S. 1947: Mathematical statistic, p. 24.

YAMAMOTO, T. 1956: On the characteristics of unit catch, with attached figures, Statistics and Survey Division, Ministry of Agricluture and Forestry, Japanese Government (Norin Suisan Chosa Siryo, No. 6) pp. 115+103.

YOSHIHARA, T. 1951: On the fitting, the summation, and an application of the logistic curve, J. Tokyo Univ. Fish. **38**, 181–195.

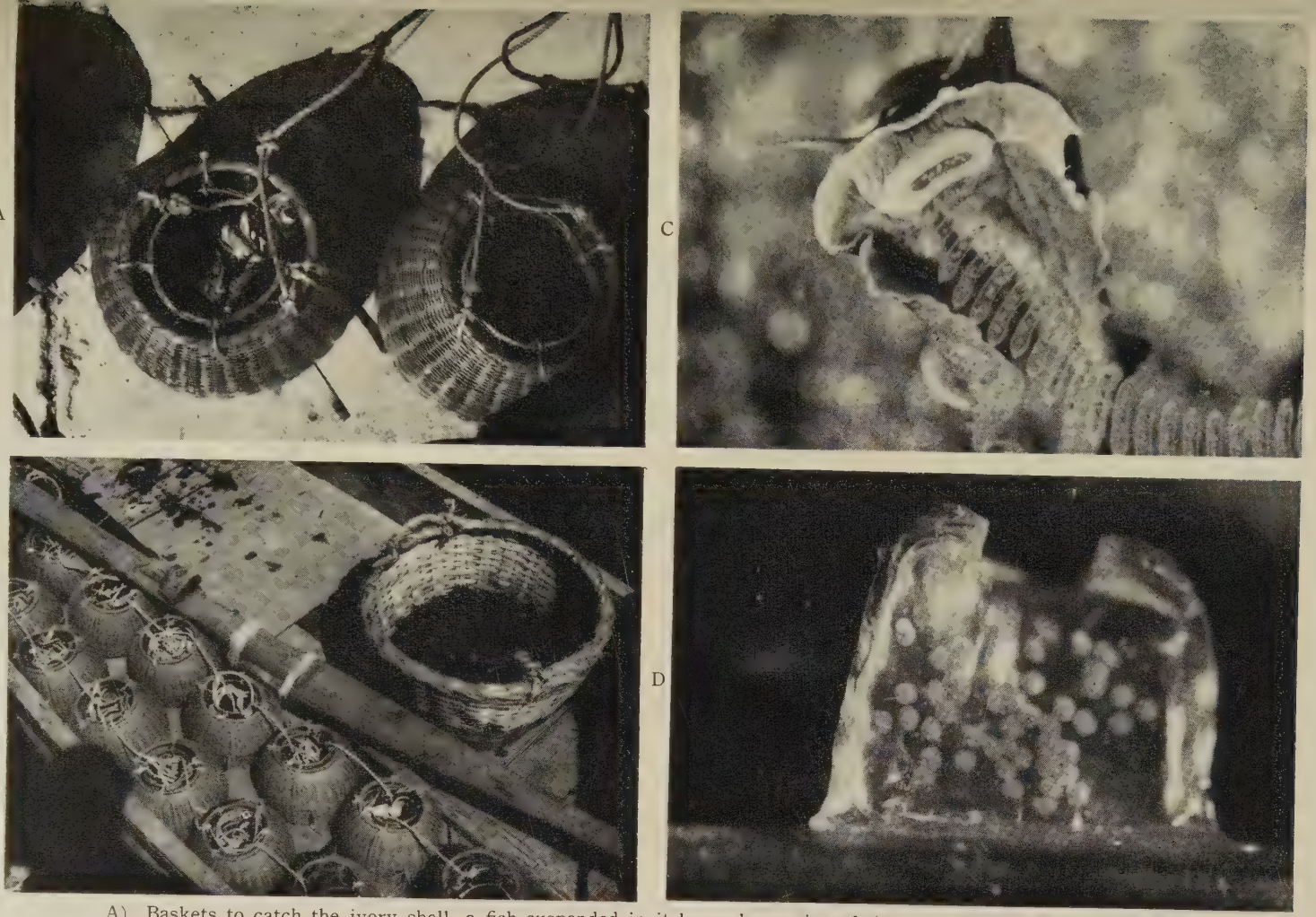
——— 1952: Effect of population-density and pond-area on the growth of fish, ibid, **39**, 47–61.

——— 1952: On the type of frequency curve of the catch, Bull. Jap. Soc. Fish. **18**, 241–244.

——— 1954: On the distribution of catches by tuna longline, J. Tokyo Univ. Fisheries, **41**, 1–26.

——— 1954: On the minimum survival rate to maintain the strength of population, read at the annual meeting of the Japanese Society of Scientific Fisheries held at Tokyo, May.

——— 1955: Methods of estimating the abundance of animal population I., II., Japanese J. Ecology **4**, 177–182, **5**, 37–41.



A) Baskets to catch the ivory shell, a fish suspended in it by a skewer is a bait. B) The catch on the boat with baskets. C) A female shell laying eggs in a glass container. D) An egg sack, which eggs are contained. Photographs C) and D) were reproduced from a film "Ecology of Shell" by the Shizen Kagaku Eigasha, Tokyo by the kind permission of Mr. T. FUKUDA, Managing Director.

Journal of the Tokyo University of Fisheries, Vol. 43, No. 2, 1957

A QUANTITATIVE STUDY ON CRUSTACEAN BOTTOM EPIFAUNA OF TOKYO BAY

Itsuo KUBO and Eiji ASADA

(Received Feb. 15, 1957)

Tokyo Bay is about 1187 km^2 in area, 16.6 km^3 in volume, 13.9 m deep on an average, and 52 m in the greatest depth. It becomes gradually deeper towards its entrance. Most parts of its basin are simple, although some elevations and depressions are present on the region near its entrance.

This bay is provided with many large cities, Tokyo, Yokohama, Kawasaki, and others, especially on its west and north-west edges. So that the waters near those cities are considerably contaminated in considerable extent, and the natural resources supporting the fisheries of the bay are being under motal effect of the drainage.

To know the present qualitative and quantitative state of bottom epifauna of this bay, and effect due to pollution of the dranage for the fauna, quantitative surveys were carried out three times during the season from May to December of 1954 using a small sized beam trawl specially prepared for the sampling of the present surveys in association with Fishery Laboratory of Faculty of Agriculture of Tokyo University, Tōkai Regional Fisheries Research Laboratory, and Fishery Biological Laboratory of our university. General account of the result obtained by the surveys will be given later elswhere by Dr. T. Ino and his collaborators of the Tōkai Regional Fisheries Research Laboratory.

In this article, some findings resulted from analyses dealt with crustacean material which was taken by the surveys, and some discussions are given. With regard to benthic communities of Tokyo bay, investigations were made already by MASUI (1943), KAWAGUCHI (1946), and SHIMADZU and YAMANE (1949) using Ekman-type bottom sampler. Among our findings, some ones agree well and some ones disagree with those made by those previous investigators. Discussions of such matters will be given later on.

Acknowledgement is made to Drs. T. INO, T. KAWANA, Y. MATSUE, Y. OSHIMA and Messrs. T. KOYAMA (captain of TAKAMARU), T. NONAKA, T. OKUTANI, J. SAGARA, K. TAKAGI. TAKETOMI, and J. TANAKA. They took part in the collection of samples and extended kindness to us in various ways. Hearty thanks are also due to Mr. T. SHIMADZU, a technician of the Freshwater Fisheries Research Laboratory, in matter of important literature, and due to Mr. S. HORI, who assisted us in preparation of manuscript of this paper. Finally it should be noted herewith that this investigation was supported by the grant in aid for fundamental scientific research of the Ministry of Education.

(1) Sampling and its method

Sampling was made three times during the time from May to December of 1954, by using the Takamaru, a ship of 10.56 gross tons with 40 H.P. oil engine, belonging to the Tokai Regional Fisheries Research Laboratory. The first sampling was undertaken on the days from May 27 to June 1, the second from August 30 to September 5, and the third on the days from 7 to 12 of December. The trawl employed for collection of the samples, on which the present paper is embodied, is with a mouth, 2.2 m in width and 0.8 m in height. The mesh of the gear, from knot to knot measures 7 mm in landing part and 18 mm in the part near its mouth. Sampling was rendered on 53 stations of the bay including Uraga channel as shown in the figure 1. In each station, one haul was made for sampling. In one haul, the trawl was pulled nearly 250 m during 5 minutes. Accordingly it becomes as follows:—the trawl sweeps about 550 m^2 ($=2.2\,m \times 250\,m$) of bottom surface as being that the lower width of the mouth of the gear runs 2.2 m as written above. In facility we used this area as an unit of comparision. So that it is that the numerals given later of this paper show the number or weight in g of animals obtained by one haul of the trawl from the unit area said above.

The efficiency of this gear for each animal is unknown. But we have done an experimint to know it in regard to a starfish, *Asterias amurensis* by means of the following method, namely one man swims attaching to the mouth of the trawl by aid of an aqualung or scuba counting the starfishes lying in front of the mouth of the trawl. Hence we can know the total number of the starfishes being actually distributed on sea bottom, and the number of the animals secured by the trawl used for collection. Wherewith the efficiency (f) in per cent may be known as $f=B/A\times100$, A is the total number of the starfish known by count made at under water, and B is that of the animals caught by the trawl. The efficiency, f taken by the experiments carried out in a place of sandy bottom, 7—9 m deep, off Kisarazu, Chiba prefecture, is 17.4% on an average.

(2) Sampling stations and those bottom characters

According to SHIMADZU and YAMANE (1948), Tokyo bay is spritable into two upper and lower parts with regard to its bottom characters. The upper or northern part is the area upper than the line of Honmoku (near Yokohama) and Kisaradu. The bottom of this part is composed of fine mud. The other part (southern part) is the area lower than the line said above. In this area, the bottom chiefly consists of sandy mud.

These two parts, upper and lower, and the part belonging to the north eastern part of the Uraga channel will be called area-A, -B, and -C respectively (Fig. 1, A, B, C).

Of all those 53 stations, the first 32 stations (1—61) belong to the area-A, and the second 11 stations (63—82) to the area-B, and the rest, 10 stations (83—92) to the area-C. In the stations, 83—89 and 92, the bottom is of sand, but it is of rock and sand in the station 90, and of rock in the station 91.

Depths in m, water temperature, transprency, chlorinity, and bottom

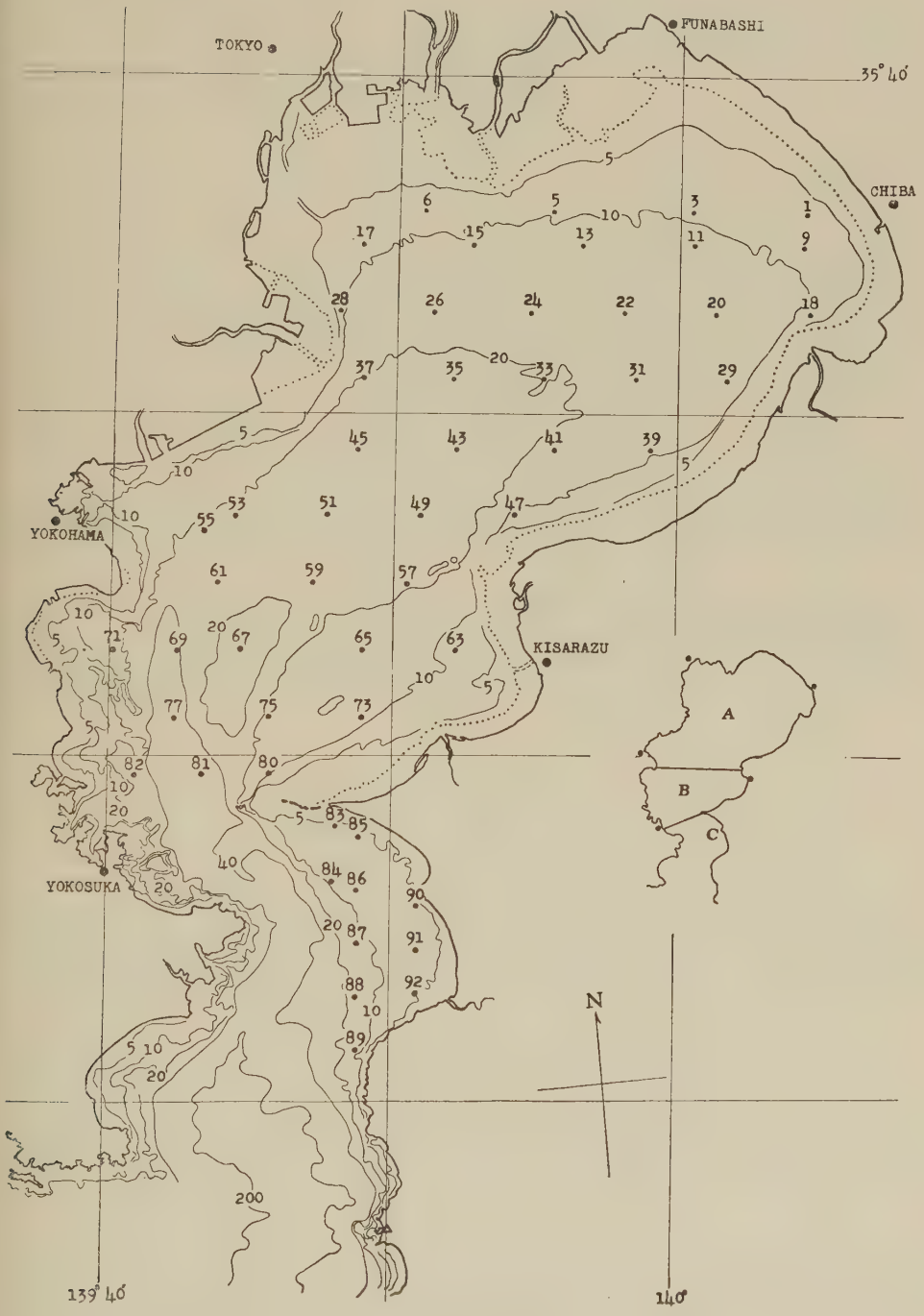


Fig. 1. Showing places of the stations, on which the samplings of this study were made, and sections (A, B and C) used in this thesis.

characters observed in the present inspection in each station, on which collection

Table 1. Depth, surface water temperature (S chlorinity (*, surface; **, bottom), bottom character (B

	Items	Number of station											
		1	3	5	6	9	11	13	15	17	18	20	22
First sampling	Depth (m)	6.0	8.0	9.0	8.0	7.0	15.0	15.0	10.0	7.0	11.0	15.0	18.0
	SWT (°C)	18.2	17.9	17.2	18.1	18.1	18.5	18.2	18.4	18.4	17.8	18.1	17.9
	BWT (°C)	15.6	15.6	16.1	—	16.1	17.5	16.4	17.5	16.8	16.5	16.5	17.0
	Transp. (m)	2.5	1.8	—	1.9	2.3	2.5	2.0	1.6	1.9	2.1	2.2	2.1
	Cl′ (‰)*	16.54	16.35	16.11	13.60	16.63	16.64	16.47	16.08	15.79	17.12	16.70	16.45
	Cl′ (‰)**	16.74	16.58	17.60	—	17.04	16.83	16.84	16.14	17.66	17.92	18.11	17.70
	BC	M	M	M	M	M	M	M	M	M	M	M	M
Second sampling	Depth (m)	10.0	12.5	9.5	9.0	11.5	14.0	15.0	10.5	11.0	12.0	15.0	19.0
	SWT (°C)	24.8	25.2	24.8	24.8	24.8	25.1	25.2	24.5	24.6	24.8	25.0	24.9
	BWT (°C)	22.6	22.0	22.5	22.2	22.6	21.5	21.0	21.6	21.1	22.3	21.5	20.2
	Transp. (m)	1.5	2.0	2.0	1.5	1.5	2.0	1.9	1.7	1.5	1.8	1.9	2.0
	Cl (‰)*	17.90	16.92	16.10	14.75	16.87	17.02	16.37	13.33	12.43	17.22	16.89	15.45
	Cl′ (‰)**	18.31	18.70	18.40	18.41	18.52	18.73	18.82	18.96	18.64	18.57	18.68	18.88
	BC	M	M	M	M	M	M	M	M	M	M	M	M
Third sampling	Depth (m)	10.0	10.0	10.0	9.0	10.0	10.0	13.0	13.0	8.0	9.0	14.0	16.0
	SWT (°C)	13.2	13.2	12.8	12.4	13.2	13.2	13.2	12.9	12.6	13.2	13.2	13.0
	BWT (°C)	13.1	13.3	13.5	14.6	13.3	13.4	14.0	14.8	14.3	13.6	12.8	13.2
	Transp. (m)	4.0	4.0	2.5	3.0	3.5	4.2	3.0	2.7	—	4.0	4.0	3.5
	Cl′ (‰)*	16.89	17.35	17.08	16.01	16.86	17.27	17.36	16.32	14.88	16.99	17.07	16.94
	Cl′ (‰)**	17.05	17.45	17.47	17.79	16.94	17.46	17.36	17.71	17.76	17.43	16.98	17.77

	Items	Number of station											
		53	55	57	59	61	63	65	67	69	71	73	75
First sampling	Depth (m)	40.0	40.0	20.0	26.0	24.0	9.0	16.8	17.5	40.0	20.0	14.0	22.0
	SWT (°C)	19.4	19.3	18.3	19.1	19.3	18.8	19.4	19.2	19.0	18.8	19.5	20.4
	BWT (°C)	16.2	16.1	16.6	16.6	16.6	16.6	16.6	16.8	16.2	16.6	16.6	16.4
	Transp. (m)	2.3	2.0	2.9	2.9	2.5	2.0	2.6	3.0	3.0	1.8	2.0	2.2
	Cl′ (‰)*	15.50	15.61	17.75	16.98	—	17.12	17.29	16.17	16.70	15.76	17. 4	16.81
	Cl′ (‰)**	18.96	18.97	18.66	18.74	18.46	18.12	18.55	18.09	19.28	18.47	18.46	18.59
	BC	M	M	M	M	M	M	M	Sh	M	M	SM	S
Second sampling	Depth (m)	21.0	17.0	23.0	26.0	3.0	15.0	20.0	16.0	—	28.0	13.0	22.0
	SWT (°C)	25.7	25.8	25.9	25.6	25.8	26.3	21.5	25.5	—	25.1	25.4	25.0
	BWT (°C)	20.3	22.2	21.9	19.3	19.8	23.7	22.1	24.2	—	21.05	23.9	22.4
	Transp (m)	2.0	1.9	3.5	2.2	2.3	2.8	3.8	3.0	—	1.8	2.9	3.5
	Cl′ (‰)*	14.21	14.32	16.99	16.52	16.39	14.86	17.66	16.68	—	16.37	17.02	16.87
	Cl′ (‰)**	18.68	18.22	18.17	18.63	18.74	18.19	18.36	17.92	—	18.42	18.12	18.32
	BC	M	M	S	M	M	S	S	S	—	M	S	S
Third sampling	Depth (m)	20.0	13.0	20.0	30.0	33.0	6.0	15.0	20.0	20.0	30.0	10.0	20.0
	SWT (°C)	13.5	12.7	13.3	13.3	13.3	13.5	13.4	13.1	13.4	14.2	13.3	14.0
	BWT (°C)	14.9	14.6	15.0	15.2	16.4	14.8	15.1	15.0	15.9	16.0	13.9	15.0
	Transp. (m)	4.0	3.5	5.9	5.3	4.8	4.8	3.5	4.5	4.8	5.0	5.0	4.5
	Cl′ (‰)*	16.85	16.27	17.10	17.18	17.75	17.66	17.46	17.57	17.65	17.80	17.57	17.82
	Cl′ (‰)**	18.75	17.85	18.05	18.01	18.30	17.71	17.99	18.04	18.37	18.34	18.04	18.24

was made, are listed on the Table 1.

WT), bottom water temperature (BWT), transparency,
C) of each station. M, mud; S, sand; Sh, shell; R, rock.

Number of station

24	26	28	29	31	33	35	37	39	41	43	45	47	49	51
18.0	20.0	15.0	17.0	20.0	30.0	20.0	12.0	18.0	27.0	30.0	20.0	10.0	30.0	38.0
17.9	17.6	17.4	17.3	17.7	18.2	18.2	18.1	18.5	18.5	18.0	18.5	18.6	18.7	18.8
16.5	16.4	17.5	16.7	16.7	16.3	16.3	16.5	16.4	16.2	16.0	16.0	16.5	16.3	16.4
2.3	2.5	1.5	2.3	2.6	—	3.0	1.6	2.2	3.1	3.0	2.5	2.4	3.0	2.8
16.35	14.35	13.72	17.66	17.25	17.13	17.03	14.65	16.99	17.00	16.90	13.39	17.13	17.21	17.25
18.09	18.50	17.41	18.11	18.40	18.70	18.79	18.64	18.68	18.84	18.83	18.85	18.48	18.85	18.94
M	M	MSh	M	—	M	M	M	SM	M	M	MSh	SM	M	M
19.0	17.0	9.0	13.0	17.0	22.0	20.0	15.0	11.0	18.0	27.0	24.0	20.0	25.0	29.0
24.7	24.7	24.6	24.8	24.9	23.8	24.6	25.4	26.2	26.0	26.0	24.3	25.8	26.0	25.6
19.7	19.6	22.9	21.9	21.8	21.2	20.85	21.8	22.8	22.5	20.9	19.7	22.8	20.6	21.6
2.0	2.4	1.8	1.8	2.0	2.4	2.3	2.3	2.0	2.4	2.3	1.8	2.1	2.2	2.3
15.07	15.59	14.05	17.22	16.52	16.66	16.60	—	16.26	16.60	—	15.68	16.28	16.24	16.46
18.80	18.78	17.68	18.63	18.75	18.69	18.69	18.58	18.36	18.33	18.66	18.82	18.38	18.57	18.74
M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
16.0	17.0	9.0	15.0	18.0	20.0	23.0	20.0	10.0	26.0	—	24.0	13.0	25.0	12.0
13.1	13.2	12.0	13.3	12.9	12.7	12.7	12.2	13.1	12.8	12.8	12.9	14.3	12.8	13.3
14.0	14.4	—	14.6	15.5	15.2	15.3	14.8	14.1	15.7	15.6	14.8	14.3	15.8	14.7
3.5	3.0	1.5	4.2	3.7	3.7	3.5	2.0	3.7	3.5	3.3	4.4	4.2	4.8	5.0
17.07	17.21	13.00	17.35	17.22	17·06	16.89	14.57	17.15	17.08	17.78	16.19	17.54	16.99	16.78
17.72	17.81	—	17.97	18.24	18.19	18.22	18.00	17.72	18.29	18.21	18.05	17.72	18.33	17.56

Number of station

77	80	81	82	83	84	85	86	87	88	89	90	91	92
47.0	20.5	46.0	17.0	9.1	10.0	7.0	10.0	40.0	30.0	13.0	7.0	7.0	7.0
18.8	18.8	18.6	19.0	17.6	17.5	17.5	17.5	17.5	17.5	17.8	18.4	18.4	17.8
16.2	16.3	16.2	16.9	17.1	17.2	17.3	17.3	17.2	17.5	17.9	18.2	18.0	17.8
2.7	2.6	2.5	2.0	3.0	2.3	3.0	2.6	3.0	3.0	2.9	2.6	2.6	3.0
16.85	17.52	17.01	16.74	17.95	—	17.82	18.13	18.29	18.07	17.83	—	17.90	17.98
18.97	18.90	18.94	18.49	18.38	—	18.16	18.40	19.08	18.74	19.12	—	18.24	18.21
M	S	M	M	S	S	S	S	S	RS	R	R	RS	S
54.0	12.0	50.0	—	7.5	11.0	7.8	4.2	18.0	27.0	23.0	8.0	9.0	7.0
24.9	24.9	24.9	25.6	26.0	24.0	26.5	26.3	26.9	27.2	26.8	27.2	27.4	27.0
17.3	24.2	16.8	—	21.1	23.8	23.7	23.8	22.0	21.9	21.9	25.9	25.9	26.3
2.3	3.2	3.0	—	4.4	—	4.5	4.2	3.4	2.5	5.5	2.6	2.9	1.9
16.22	17.07	16.89	16.34	17.96	18.36	17.87	17.92	17.67	17.82	18.29	17.95	17.85	17.77
18.84	17.97	19.12	—	18.60	18.43	18.57	18.41	18.57	18.51	18.65	18.16	18.27	18.11
M	S	MS	Sh	S	S	S	S	S	S	S	RS	R	S
22.0	10.0	45.0	10.0	5.0	—	6.0	7.0	10.0	—	—	6.0	—	—
13.9	13.9	13.8	12.3	14.8	15.8	14.7	15.8	13.6	14.8	14.0	13.8	13.5	13.2
15.9	14.3	16.0	13.3	15.9	17.1	14.9	16.6	15.9	16(0	16.0	14.6	15.2	15.2
4.6	3.8	4.8	3.5	4.2	8.0	3.9	7.2	—	—	—	4.5	5	—
17.79	18.05	17.78	17.02	17.90	18.31	18.21	18.49	17.84	18.10	18.11	18.08	17.64	18.05
18.34	17.99	18.38	16.92	18.14	18.56	18.24	18.49	18.33	18.34	18.54	18.11	18.23	18.30

(3) Number of species and faunal closeness

(a) Number of species

As mentioned already, sampling of specimens was carried out three times, the first in late spring, the second in late summer, and the third in late fall. The species procured throughout all those samplings are 84 in number. Among those, each one species is referable to stomatopoda and amphipoda respectively, five species to isopoda, 21 species to macrura, 10 ones to anomura, and 46 ones to brachyura. Those specific names will be listed in the following lines.

(a) Stomatopoda

(1) *Squilla oratoria* De HAAN.

(b) Isopoda

(1) Isopoda sp.
(2) *Cirolana japonensis* (RICHARDSON).
(3) *Cleantis planicauda* (BENEDICT).
(4) *Idotea metallica* BOSC.
(5) *Idotea* sp.

(c) Amphipoda

(1) Amphipoda sp.

(d) Macrura

(1) *Trachypenaeus curvirostris* (STIMPSON).
(2) *Metapenaeopsis acclivis* (RATHBUN).
(3) *Metapenaeopsis dalei* (RATHBUN).
(4) *Eusicyonia cristata* (De HAAN).
(5) *Leptochela gracilis* STIMPSON.
(6) *Leptochela aculeocaudata* PAULSON.
(7) *Plesionika* sp.
(8) *Crangon japonicus* (MIERS).
(9) *Crangon rapax* (De HAAN).
(10) *Crangon bis-incisus* (De HAAN).
(11) *Spirontocaris pectinifera* (STMPSON).
(12) *Heptacarpus rectirostris* (STIMPSON).
(13) *Heptacarpus minutus* (YOKOYA).
(14) *Latreutes planirostris* (De HAAN).
(15) *Lysmata* (*Hippolysmata*) *vittata* (STIMPSON).
(16) *Birulia kishinouyei* (YOKOYA).
(17) *Palaemon* (*Palaemon*) *macrodactylus* (RATHBUN).
(18) *Palaemon* (*Palaemon*) *serrifer* (STIMPSON).
(19) *Processa japonic*a (De HAAN).
(20) *Crago affinis* (De HAAN).
(21) *Sclerocrangon intermedius* STIMPSON.

(e) Anomura

(1) *Galathea elegans* WHITE.

(2) *Galathea orientalis* Stimpson.
(3) *Porcellanella picta* Stimpson.
(4) *Clibanarius bimaculatus* (De Haan).
(5) *Paguristes digitalis* Stimpson.
(6) *Diogenes edwardsii* (De Haan).
(7) *Eupagrus constans* Stimpson.
(8) *Eupagrus trigonocheirus* Stimpson.
(9) Hermit-crab sp. (1).
(10) Hermit-crab sp. (2).

(f) Brachyura

(1) *Paradromia japonica* (Henderson).
(2) *Latreillia phalangium* De Haan.
(3) *Dorippe granulata* De Haan.
(4) *Nursia elegans japonicus* Sakai.
(5) *Arcania undecimspinosa* De Haan.
(6) *Leucosia longifrons* De Haan.
(7) *Philyra platycheira* De Haan.
(8) *Achaeus japonicus* De Haan.
(9) *Achaeus tuberculatus* Miers.
(10) *Achaeus stenorhynchus* Rathbun.
(11) *Pugettia quadridens* De Haan.
(12) *Pugettia incisa* (De Haan).
(13) *Pugettia minor* Ortmann.
(14) *Pugettia nipponensis* Rathbun.
(15) *Pugettia* sp.
(16) *Hyastenus diacanthus* (De Haan).
(17) *Hyastenus elongatus* Ortmann.
(18) *Scyra compressipes* Stimpson.
(19) *Pisoides ortmanni* (Balss).
(20) *Chorilia longipes japonicus* (Miers).
(21) *Micippa thalia* (Herbst).
(22) *Lambrus* (*Platylambrus*) *validus* De Haan.
(23) *Parthenopoides pteromerus* Ortmann.
(24) *Lissocarcinus laevis* Miers.
(25) *Charybdis bimaculata* (Miers).
(26) *Charybdis japonica* (A. Milne-Edwards).
(27) *Neptunus* (*Hellenus*) *hastatoides* (Fabricius).
(28) *Neptunus sanguinolentus* (Herbst).
(29) *Neptunus* (*Amphitrite*) *gladiator* (Fabricius).
(30) *Thalamita oculea* Alcock.
(31) *Kraussia integra* De Haan.
(32) *Cancer gibbosulus* (De Haan).
(33) *Cancer pygmaeus* Ortmann.
(34) *Actaea savignyi* (H. Milne-Edwards).
(35) *Xanthias elegans* (Stimpson).

(36) *Pilumnus minutus* De HAAN.
(37) *Actumnus squamosus* (De HAAN).
(38) *Pilumnoplax vestita* (De HAAN).
(39) *Homoioplax haswelli* (MIERS).
(40) *Pinnotheres cardii* BÜRGER.
(41) *Pinnixa rathbuni* SAKAI.
(42) *Astenognathus inaequipes* STIMPSON.
(43) *Hemigrapsus penicillatus* (De HAAN).
(44) Brachyura sp. 1.
(45) Brachyura sp. 2.
(46) Brachyura sp. 3.

Among those species enumerated above, *Leptochela aculeocaudata*[4], *Heptacarpus minutus*, and *Plesionika* sp. are new to this bay. Details of the results of each sampling are summarized in the Table 2. As seen on the Table 2, brachyura are most throughout all seasons. Macrura, anomura, and isopoda are in the order in which they are numerous. Stomatopoda are least, represented by only one species.

Table 2. Number of species caught in each sampling, and total number of individuals collected (Tn), and total weight in g of all the specimens dealt with (Tw).

Sampling	Number of species					Total	Tn	Tw
	Stomatopoda	Macrura	Anomura	Brachyura	Others			
1st	1	17	7	35	0	60	3528	3561.5
2nd	1	13	5	23	4	46	1052	2109.5
3rd	1	16	6	23	3	49	2043	1068.4
1st–3rd	1	21	10	46	6	84	6623	6739.4

Table 3. Average number of species per unit area of each sampling and area. (a), excluding the species, of which station unknown; (b) including those.

Area	Sampling			
	1st	2nd	3rd	1st–3rd
A	3.3	2.3[(a)]	2.3	4.6
B	10.4	6.6[(a)]	9.8	18.8
C	4.3	3.5[(a)]	2.8	9.3
A–C	5.1	3.6[(b)]	4.0	4.3

Distribution of number of species per unit area of each station and each sampling is given on the Tables 12–14 and Figs. 3A–6A. Average number of species per unit area of each sampling and of each area as shown on the Table 3. As seen

on the Table 3, average number of species per unit area considerably differs by area as well as by season. Of those three areas, the area-B is most plentiful on species. The area-C outnumbered the area-A at all seasons. The average number of species per unit area is 4.6, 18.8, and 9.3 on areas-A, -B, and -C respectively, and that is most at late spring, the next at late fall, and least at late summer. No difference is, however, seen of the same item on east and west halves of the bay.

(b) Faunal closeness between areas

A number of methods of notation on degree of faunal closeness between two localities has been known by many[1] investigators. In the present study, we have, however, used ŌTUKA's coefficient of closeness[8], $p = c/\sqrt{S_1 S_2}$, because of it affords most neutral values. Where S_1 and S_2 are the number of species of any given two areas, and c is the number of species common to those two given areas.

Number of species per unit area of stomatopoda and decapoda of each sampling and area, as well as number of species common to any given two areas are enumerated on the Table 4. Concrete specific names of the common species are given in the following lines.

Common species to the areas-A and -B at the first sampling are:—

(1) *Squilla oratoria.*
(2) *Crago affinis.*
(3) *Crangon japonicus.*
(4) *Trachypenaeus curvirostris.*
(5) *Latreutes planirostris.*
(6) *Heptacarpus minutus.*
(7) *Plesionika* sp.
(8) *Porcellanella picta.*
(9) *Arcania undecimspinosa.*
(10) *Charybdis bimaculata.*
(11) *Pinnixa rathbuni.*
(12) *Pilumnoplax vestita.*

Species common to the areas-A and -C at the first sampling are:—

(1) *Squilla oratoria.*
(2) *Crago affinis.*
(3) *Metapenaeopsis acclivis.*
(4) *Heptacarpus minutus.*
(5) *Latreutes planirostris.*
(6) *Charybdis bimaculata.*

Species common to the areas-B and -C at first sampling are:—

(1) *Squilla oratoria.*
(2) *Metapenaeopsis dalei.*
(3) *Latreutes planirostris.*
(4) *Heptacarpus minutus.*
(5) *Crago affinis.*
(6) *Galathea orientalis.*
(7) *Eupagrus constans.*
(8) *Charybdis bimaculata.*
(9) *Cancer gibbosulus.*
(10) *Nursia elegans japonica.*
(11) *Pilumnus minutus.*
(12) *Actumus squamosus.*

Species common to the areas-A and -B at the second sampling are:—

(1) *Squilla oratoria.*
(2) *Crangon japonicus.*
(3) *Latreutes planirostris.*
(4) *Crago affinis.*
(5) *Porcellanella picta.*
(6) *Pilumnoplax vestita.*
(7) *Charybdis bimaculata.*
(8) *Arcania undecimspinosa.*

Species common to the areas-A and -C at the second sampling are:—

(1) *Squilla oratoria.*
(2) *Crangon japonicus.*
(3) *Latreutes planirostris.*
(4) *Crago affinis.*
(5) *Arcania undecimspinosa.*
(6) *Charybdis bimaculata.*

Species common to the areas-B and -C at the second sampling are:—

(1) *Squilla oratoria.*
(2) *Trachypenaeus curvirostris.*
(3) *Latreutes planirostris.*
(4) *Heptacarpus rectirostris.*
(5) *Heptacarpus minutus.*
(6) *Crangon japonicus.*
(7) *Crago affinis.*
(8) *Galathea orientalis.*
(9) *Charybdis bimaculata.*
(10) *Arcania undecimspinosa.*
(11) *Pilumnus minutus.*
(12) *Pugettia incisa.*
(13) *Pugettia quadridens.*

Species common to the areas-A and -B at the third sampling are:—

(1) *Squilla oratoria.*
(2) *Trachypenaeus curvirostris.*
(3) *Crangon japonicus.*
(4) *Heptacarpus minutus.*
(5) *Crago affinis.*
(6) *Porcellanella picta.*
(7) *Charybdis bimaculata.*
(8) *Pilumnoplax vestita.*

Species common to the areas-A and -C at the third sampling are:—

(1) *Trachypenaeus curvirostris.*
(2) *Heptacarpus minutus.*
(3) *Crago affinis.*
(4) *Porcellanella picta.*

Species common to the areas-B and -C at the third sampling are:—

(1) *Trachypenaeus curvirostris.*
(2) *Heptacarpus minutus.*
(3) *Latreutes planirostris.*
(4) *Crago affinis.*
(5) *Porcellanella picta.*
(6) *Diogenes edwardsii.*
(7) *Neptunus hastatoides.*
(8) *Pugettia incisa.*
(9) *Actumnus squamosus.*
(10) *Pilumnus minutus.*

Species common to the areas-A and -B throughout the first-third samplings are:—

(1) *Squilla oratoria.*
(2) *Trachypenaeus curvirostris.*
(3) *Metapenaeopsis acclivis.*
(4) *Crangon japonicus.*
(5) *Latreutes planirostris.*
(6) *Heptacarpus minutus.*
(7) *Plesionika* sp.
(8) *Crago affinis.*
(9) *Porcellanella picta.*
(10) *Pilumnoplax vestita.*
(11) *Charybdis bimaculata.*
(12) *Pinnixa rathbuni.*
(13) *Arcania undesimspinosa.*

Species common to the areas-A and -C through the first-third samplings are:—

(1) *Squilla oratoria.*
(2) *Trachypenaeus curvirostris.*
(3) *Metapenaeopsis acclivis.*
(4) *Crangon japonicus.*
(5) *Latreutes planirostris.*
(6) *Heptacarpus minutus.*
(7) *Plesionika* sp.
(8) *Crago affinis.*
(9) *Porcellanella picta.*
(10) *Pilumnoplax vestita.*

(11) *Charybdis bimaculata.*
(12) *Pinnixa rathbuni.*
(13) *Arcania undesimspinosa.*

Species common to the areas-A and -C throughout the first-third samplings are:—

(1) *Squilla oratoria.*
(2) *Trachypenaeus curvirostris.*
(3) *Metapenaeopsis acclivis.*
(4) *Crangon japonicus.*
(5) *Latreutes planirostris.*
(6) *Heptacarpus minutus.*
(7) *Crago affinis.*
(8) *Porcellanella picta.*
(9) *Charybdis bimaculata.*
(10) *Arcania undesimspinosa.*

Species common to the areas-B and -C throughout the first-third samplings are:—

(1) *Squilla oratoria.*
(2) *Trachypenaeus curvirostris.*
(3) *Metapenaeopsis acclivis.*
(4) *Metapenaeopsis dalei.*
(5) *Crangon japonicus.*
(6) *Heptacarpus rectirostris.*
(7) *Heptacarpus minutus.*
(8) *Latreutes planirostris.*
(9) *Crago affinis.*
(10) *Galathea orientalis.*
(11) *Diogenes edwardsii.*
(12) *Clibanarius bimaculatus.*
(13) *Porcellanella picta.*
(14) *Eupagurus constans.*
(15) *Eupagurus trigonocheirus.*
(16) *Charybdis bimaculata.*
(17) *Neptunus minutus.*
(18) *Arcania undecimspinosa.*
(19) *Pugettia incisa.*
(20) *Pugettia quadridens.*
(21) *Pugettia minor.*
(22) *Dorippe granulata.*
(23) *Cancer gibbosulus.*
(24) *Nursia elegans japonica.*
(25) *Actumnus squamosus.*
(26) *Micippa thalia.*
(27) *Pilumnus minutus.*
(28) *Pisoides ortmanni.*
(29) Brachyura sp.

Moreover species common to all the areas-A, -B, and -C are:—

(1) *Squilla oratoria.*
(2) *Trachypenaeus curvirostris.*
(3) *Metapenaeopsis acclivis.*
(4) *Crangon japonicus.*
(5) *Latreutes planirostris.*
(6) *Heptacarpus minutus.*
(7) *Crago affinis.*
(8) *Porcellanella picta.*
(9) *Charybdis bimaculata.*
(10) *Arcania undecimspinosa.*

The coefficient (p) in each combination of any given two areas is put on he Table 5. As shown on the table, it is larger in order of the combinations-AB, BC, and -AC in the first and second samplings, but it is larger in order of the ombinations-BC, -AB, and -AC in the third one respectively. Whilst it is 0.59, .43, and 0.34 in combinations-BC, -AB, and -AC respectively in the data com- ined those of all three samplings. The trend found in the combined data is the me as is the case with the third sampling. According to the facts given above, may be said that the area-B is nearer to the area-C than to the area-B a least the crustacean fauna.

So far as our investigation goes, each area is characterized by the species iven below. Differential species are 5 in number: *Pinnixa tumida, Pinnotheres ardii, Chorilia longipes japonicus, Hemigrapsus penicillatus, Homoioplax haswelli*

Table 4. Number of species of stomatopoda and decapoda collected at each sampling and area, and number of species common to each given two areas.

Area	Number of species				Combi-nations	Common species			
	1st	2nd	3rd	1st–3rd		1st	2nd	3rd	1st–3rd
A	15	9	11	18	AB	12	8	8	13
B	40	28	40	50	AC	6	6	4	10
C	31	24	14	48	BC	12	13	10	28

Table 5. Coefficient of faunal closeness between two areas, $p=c/\sqrt{S_1 S_2}$ (See text). C_b, combination; I–III, first-third samplings; A–C, areas-A~C.

C_b	p	C_b	p	C_b	p	C_b	p
I_{AB}	0.49	II_{AB}	0.50	III_{AB}	0.38	$I\text{–}III_{AB}$	0.43
I_{AC}	0.28	II_{AC}	0.40	III_{AC}	0.32	$I\text{–}III_{AC}$	0.34
I_{BC}	0.34	II_{BC}	0.49	III_{BC}	0.42	$I\text{–}III_{BC}$	0.57

in the area-A; are 15 species in number: *Spirontocaris pectinifera*, *Lysmata* (*Hippolysmata*) *vittata*, *Birulia kishinouyei*, *Leptochela gracilis*, *Leptochela aculeocaudata*, *Palaemon serrifer*, *Sclerocrangon intermedius*, *Paradromia japonica*, *Achaeus tuberculatus*, *Scyra compressipes*, *Leucosia longifrons*, *Actaea savignyi*, *Parthenopoides pteromerus* in the area-B; are 18 ones in number: *Eusicyonia cristata*, *Crangon bis-incisus*, *Crangon rapax*, *Processa japonica*, *Galathea elegans*, *Lissocarcinus laevis*, *Kraussia integra*, *Lambrus validis*, *Hyastenus elongatus*, *Hyastenus diacanthus*, *Latreilla phalangium*, *Achaeus japonicus*, *Neptunus* (*Amphitrite*) *gladiator*, *Thalamita oculea*, *Xanthias elegans*, *Charybdis japonica*, *Philyra platycheira* in the area-C.

As known from the mentions given above, there is a tendency parallel to that open-sea forms occur much as the areas go southwards. This tendency is parallel to the chlorinity of Tokyo bay and Uraga channel, in which this element becomes gradually higher towards open-sea, viz. mean chlorinity of the area-B is higher than that of the area-A, and that of the area-C is higher than that of the area-B.

(4) Individual number per unit area.

Average number of individuals per unit area of each sampling and each area is given in the Table 6 and Figs. 3B–6B. From those, it is clear that the average numbers at the first, second, and third samplings are in the order in which they are numerous, viz., in other words the average number in the late spring is larger than that in the late fall, and the average number in the late fall is larger than that in the late summer. In concrete, the average numbers are 66.6 in the late spring, 39.5 in the late autumn, and 19.9 in the late summer.

The average number per unit area of each area is larger in order of the areas-B, -A, and -C in the cases of the first and third samplings except for those

of the second one, in which the order of the areas-A and -C appears to be reversed. But the order is the same as is the case with the first and third samplings when it analysed dealing with the data combined those of all the three samplings. The individual number on average is 133.9 in the area-B, 17.8 in the area-A, and 11.6 in the area-C. Thus the density of the population appears to be much higher in the area-B than in other two areas. This tendency is entirely the same as is the case with average number of species per unit area.

Further it is revealed that the average individual number per unit area is much larger in east half of the bay in comparison to that of the west half of the bay without exception, at least, within the limit of the data dealt with herein. The number is 64.4 on the eastern half, and 30.6 on the other half as given in the Table 7.

Table 6. Average number of individuals and average weight per unit area by each sampling and area. For A–C, and I–III, see table 5.

Area	Number of individual				Weight in g			
	I	II	III	I–III	I	II	III	I–III
A	25.1	6.9	21.3	17.8	44.4	33.3	15.5	31.6
B	237.5	44.8	119.6	133.9	185.8	59.2	42.3	95.8
D	11.4	15.8	7.6	11.6	13.7	28.9	12.9	18.4
A–C	66.6	19.8	38.5	41.6	67.9	39.6	20.6	42.7

Table 7. Average number of individual per unit area (An), average weight in g per unit area (Aw), and average weight in g per individual (Wi) by sampling and by east and west halves of the bay.

Sampling	An			Aw			Wi		
	East	West	Entire	East	West	Entire	East	West	Entire
I	93.9	64.9	79.4	66.0	95.1	80.6	0.7	1.5	1.0
II	22.9	10.3	16.6	33.6	46.2	40.0	1.5	4.5	2.4
III	76.3	16.6	46.4	49.2	17.3	33.3	0.6	1.0	0.7
I–III	64.4	30.6	47.5	42.4	52.8	47.6	0.9	2.3	1.4

(5) Weight per unit area

Gravimetric data of each sampling and area are given in the Tables 15–18. Average weight per unit area of the first sampling, that of the second sampling, and that of the third one are in the sequence in which those are large in both areas-A and -B, namely the weight is largest in late spring, smallest in late autumn, and intermediate in late summer at those two areas. Since the sequens of the first and second samplings in the area-C are convers in comparison to that of the areas-A and-B, the sequence runs as follows: the second, first, and

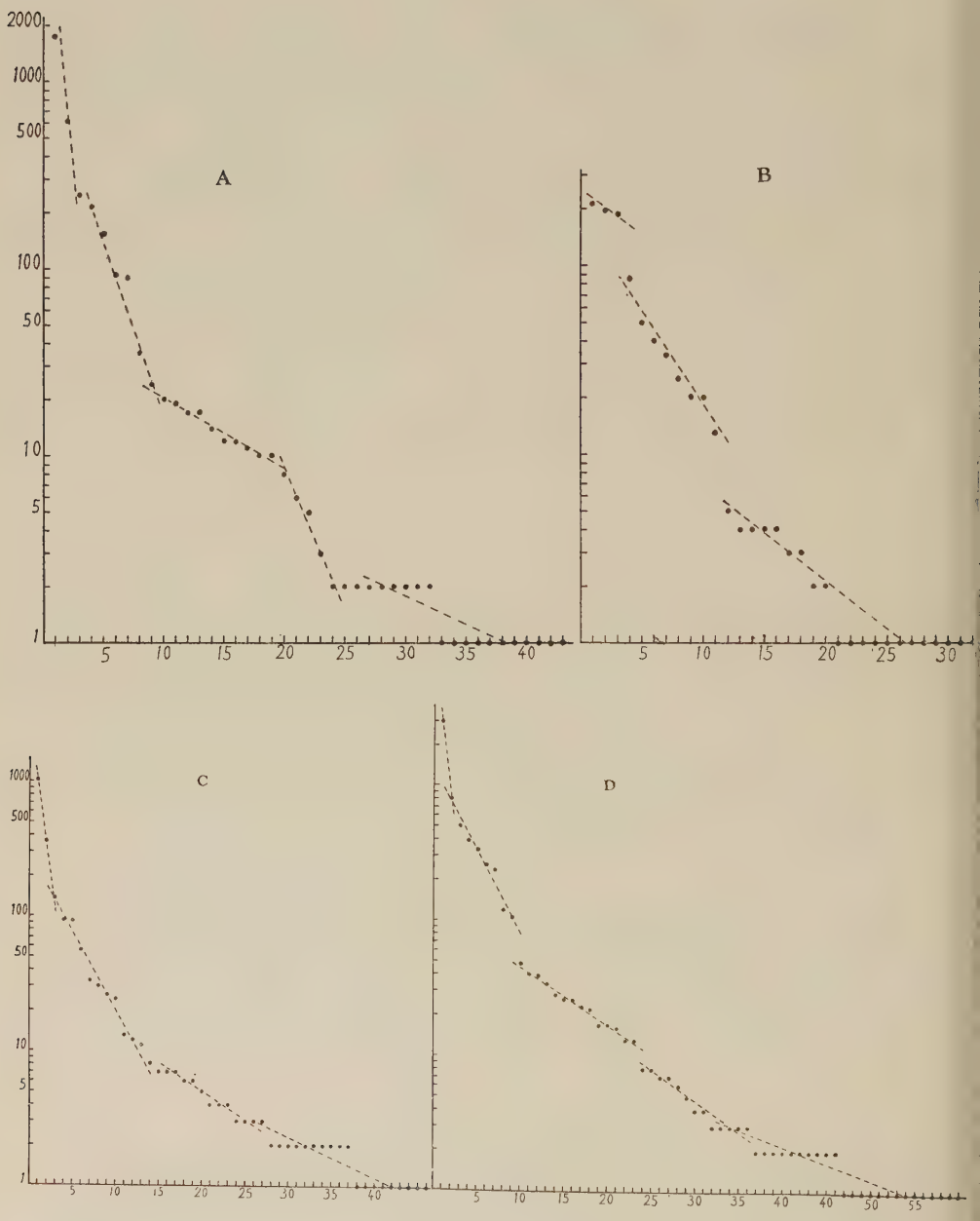


Fig. 2. Relation between logarithms of individual number (y-axis) and sequence number of component species of the animal association (x-axis). For sequence number of the component species and individual number of those species of the first sampling (A), see Table 8; for those of the second (B), see Table 9; for those of the third (C), see Table 10; for those of the total material combined all those taken by the samplings from the first to the third (D), see Table 11.

third. But in the total data combined datum made by each sampling, it runs as the first, second, and third as the average weight per unit area is being 67.9, 39.6, and 20.6 in g respectively (Table 6). This order differs from that in the case of average individual number per unit area, in which the order is put as the first, third, and second. This means nothing more or less than that the population in the time of the third sampling is numerous in individual number, but scarce in weight. The reason for this fact seems to the authors to be due to that the autumn population is composed of mostly rather young forms hatched out in late spring or summer of this year, while the summer population consists of mostly grownups born, at least, in last year, or some years ago,

Among the areas-A, -B, and -C, the area-B is the largest in the average weight per unit area in all samplings, the area-A medium, and the area-C least in the same matter. This trend is entirly equal to that found in the case with number of individuals per unit area. The average weight per unit area is 31.6 g in the area-A, 95.8 g in the area-B, and 18.4 g in the area-C. The average of the weight of the grand total is 42.7 g as seen in the Table 6.

Comparing with the average weight per unit area of both east and west halves of the bay, the average weight of the east half is much less than that of the west one as being 66.0 g in the east half, and 95.1 g in the other half in the first sampling, and 33.6, 46.2 g respectively in the second sampling. But it is conversely larger in the east half than in the west half in the third sampling, and the average weight is 49.2 g in the east half, and 17.3 g in the west half. It is of interest to note, however, that the average larger in the western half than in the eastern one. From this fact it may be supposed that the inhabitants of the west half of the bay is larger in size than those of the east half.

(6) Order of individual numbers

In some animal associations, it is known that the relationship between logarithms of individual numbers (y) of each species belonging to a certain animal association and sequence number of component species of the association arranged according to abundance of individuals of each species (x) is expressed by the formula of the law of geometrical progression of population density,

$$\log y = b - ax,$$

by Motomura[6,7]. In the present investigation, some analyses have been done on organization of crustacean association of Tokyo bay basing on the data excluding those of the Uraga channel.

The order of species basing on individual number at each sampling and the data combined all given in the Tables 8–11. The relavance between x and y of these data is plotted in Fig. 2, A—D. As seen from those figures, the relation is not simple. The regression between those items are made up of some linear lines. To know the reason of this complexity is difficult within the scope of the present study. This subject fairly begs for further investigations.

Same dominant species are found to exist in each sampling. Although some alternations in order of them have been found among the dominants of each

Table 8. List of species arranged in order of abundance, taken at the first sampling.

Sequence number	Specific name	Individual number
(1)	*Crago affinis*	1754
(2)	*Heptacarpus minutus*	612
(3)	*Pilumnoplax vestita*	247
(4)	*Crangon japonicus*	216
(5)	*Charybdis bimaculata*	153
(6)	*Trachypen. curvirostris*	92
(7)	*Heptacarpus rectirostris*	89
(8)	*Squilla oratoria*	35
(9)	*Latreutes planirostris*	24
(10)	*Eupagurus constans*	20
(11)	*Birulia kishinouyei*	19
(12)	*Spirontocaris pectinifera*	17
(13)	*Porcellanella picta*	17
(14)	*Arcania undecimspinosa*	14
(15)	*Pilumnus minutus*	12
(16)	*Sclerocran. intermedius*	12
(17)	*Metapenaeopsis dalei*	11
(18)	*Plesionika* sp.	10
(19)	*Scyra compressipes*	10
(20)	*Eupag. trigonocheirus*	8
(21)	*Paradromia japonica*	6
(22)	*Pugettia incisa*	5
(23)	*Chorilia long. japonicus*	3
(24)	*Galathea orientalis*	2
(25)	*Actumnus squamosus*	2
(26)	*Metapenaeopis acclivis*	2
(27)	*Paguristes digitalis*	2
(28)	*Dorippe granulata*	2
(29)	*Cancer pygmaeus*	2
(30)	*Actaea savignyi*	2
(31)	*Pinnotheres cardii*	2
(32)	*Pinnixa rathbuni*	2
(33)	*Nursia elegans japonicus*	1
(34)	*Cancer gibbosulus*	1
(35)	*Palaemon macrodactylus*	1
(36)	*Leptochela aculeocaudata*	1
(37)	*Leucosia longifrons*	1
(38)	*Pugettia quadridens*	1
(39)	*Pugettia nipponensis*	1
(40)	*Pugettia* sp.	1
(41)	*Micippa thalia*	1
(42)	*Astenognathus inaequipes*	1
(43)	Brachyura sp.	1

Table 9. List of species arranged in order of abundance, taken at the second sampling.

Sequence number	Specific name	Individual number
(1)	*Crago affinis*	213
(2)	*Heptacarpus rectirostris*	196
(3)	*Heptacarpus minutus*	188
(4)	*Pilumnoplax vestita*	85
(5)	*Charybdis bimaculata*	49
(6)	*Squilla oratoria*	41
(7)	*Crangon japonicus*	33
(8)	*Pilumnus minutus*	25
(9)	*Trachypen. curvirostris*	20
(10)	*Spirontocaris pectinifera*	20
(11)	*Eupagurus constans*	13
(12)	*Paradromia japonica*	5
(13)	*Pugettia incisa*	4
(14)	*Neptunus sanguinolentus*	4
(15)	Amphipoda sp.	4
(16)	*Porcellanella picta*	4
(17)	*Latreutes planirostris*	3
(18)	*Birulia kishinouyei*	3
(19)	Hermit-crab 1	2
(20)	*Pinnotheres cardii*	2
(21)	*Arcania undecimspinosa*	1
(22)	*Galathea orientalis*	1
(23)	*Cirolana japonensis*	1
(24)	*Pugettia quadridens*	1
(25)	*Plesionika* sp.	1
(26)	*Leptochela aculeocaudata*	1
(27)	*Leptochela gracilis*	1
(28)	*Sclerocran. intermedius*	1
(29)	*Dorippe granulata*	1
(30)	*Achaeus tuberculatus*	1
(31)	*Scyra compressipes*	1
(32)	*Cancer pygmaeus*	1

Table 10. List of species arranged in order of abundance taken at the third sampling.

Sequence number	Specific name	Individual number	Sequence number	Specific name	Individual number
(1)	*Crago affinis*	1011	(24)	*Leptochela gracilis*	3
(2)	*Heptacarpus rectirostris*	360	(25)	*Cancer pygmaeus*	3
(3)	*Crangon japonicus*	136	(26)	*Pinnixa rathbuni*	3
(4)	*Trachypen. curvirostris*	93	(27)	Brachyura sp.	3
(5)	*Latreutes planirostris*	93	(28)	*Neptunus hastatoides*	2
(6)	*Charybdis bimaculata*	56	(29)	*Diogenes edwardsii*	2
(7)	*Squilla oratoria*	33	(30)	*Idotea* sp.	2
(8)	*Pugettia incisa*	30	(31)	*Plesionika* sp.	2
(9)	*Heptacarpus minutus*	26	(32)	*Palaemon serrifer*	2
(10)	*Leptochela aculeocaudata*	24	(33)	*Lysmata vittata*	2
(11)	*Sclerocran. intermedius*	13	(34)	Hermit-crab 2	2
(12)	Amphipoda sp.	12	(35)	*Achaeus tuberculatus*	2
(13)	*Pilumnus minutus*	11	(36)	*Pugettia quadridens*	2
(14)	*Clibanarius bimaculatus*	8	(37)	*Pugettia minor*	2
(15)	*Porcellanella picta*	7	(38)	*Actumnus squamosus*	1
(16)	*Eupagurus constans*	7	(39)	Isopoda sp.	1
(17)	*Pilumnoplax vestita*	7	(40)	*Metapenaeopsis acclivis*	1
(18)	*Metapenaeopsis dalei*	6	(41)	*Spirontocaris pectinifera*	1
(19)	*Paradromia japonica*	6	(42)	*Achaeus stenorhynchus*	1
(20)	*Scyra compressipes*	5	(43)	*Pisoides ortmanni*	1
(21)	*Galathea orientalis*	4	(44)	*Parthenop. pteromerus*	1
(22)	*Arcania undecimspinosa*	4	(45)	*Homoioplax haswelli*	1
(23)	*Pinnotheres cardii*	4	(46)	*Hemigrapsus penicillatus*	1

sampling, the species are largely the same in each sampling as given in the following lines. The dominants are 7 species set forth below in the first sampling: *Crago affinis, Heptacarpus minutus, Pilumnoplax vestita, Crangon japonicus, Charybdis bimaculatus, Trachypenaeus curvirostris,* and *Heptacarpus rectirostris*; are 6 ones: *Crago affinis, Heptacarpus rectirostris, H. minutus, Pilumnoplax vestita, Charybdis bimaculata,* and *Squilla oratoria* in the second sampling; are also 6 ones: *Crago affinis, Heptacarpus minuta, Crangon japonicus, Trachypenaeus curvirostris, Latreutes planirostris,* and *Charybdis bimaculata* in the third one. And those based on the whole material combined all samples taken at all seasons are those enumerated below: *Crago affinis, Heptacarpus minutus, H. rectirostris, Crangon japonicus, Pilumnoplax vestita, Charybdis bimaculata, Trachypenaeus curvirostris, Latreutes planirostris,* and *Squilla oratoria.* As seen above, *Crago affinis* appears to be the most dominant in crustacea of Tokyo bay. It was caught overwhelmingly throughout all seasons in comparison to other species. The second dominant species is *Heptacarpus minutus,* and the third *H. rectirostris. Crago affinis, Crangon japonicus, Pilumnoplax vestita, Charybdis bimaculata, Trachypenaeus curvirostris, Latreutes planirostris, Squilla oratoria* are found having wide and dense distribution all over the bay. Consequently it may be said that they are playing an important rôle for fish-production of the bay as an important natural diet of fishes. *Heptacarpus minutus, H. rectirostris,* and

Table 11. List of species arranged in order of abundance, taken at samplings from the first to the third.

Sequence number	Species name	Individual number	Sequence number	Species name	Individual number
(1)	*Crago affinis*	2975	(31)	*Leptochela gracilis*	4
(2)	*Heptacarpus minutus*	793	(32)	*Actumnus squamosus*	3
(3)	*Heptacarpus rectirostris*	499	(33)	*Metapenaeopsis acclivis*	3
(4)	*Crangon japonicus*	385	(34)	*Achaeus tuberculatus*	3
(5)	*Pilumnoplax vestita*	339	(35)	*Chorilia longip. japonicus*	3
(6)	*Charybdis bimaculata*	258	(36)	*Actaea savignyi*	3
(7)	*Trachypen. curvirostris*	235	(37)	*Diogenes edwardsii*	2
(8)	*Latreutes planirostris*	120	(38)	*Pugettia minor*	2
(9)	*Squilla oratoria*	105	(39)	*Dorippe granulata*	2
(10)	*Pilumnus minutus*	48	(40)	Brachyura sp. 1	2
(11)	*Eupagurus constans*	40	(41)	*Idotea* sp.	2
(12)	*Pugettia incisa*	39	(42)	*Palaemon serrifer*	2
(13)	*Spirontocaris pectinifera*	34	(43)	*Lysmata vittata*	2
(14)	*Porcellanella picta*	28	(44)	*Paguristes digitalis*	2
(15)	*Leptochela aculeocaudata*	26	(45)	Hermit-crab sp. 2	2
(16)	*Sclerocran intermedius*	26	(46)	*Parthenopo. pteromerus*	2
(17)	*Arcania undecimspinosa*	23	(47)	*Cirolana japonensis*	1
(18)	*Birulia kishinouyei*	22	(48)	Isopoda sp.	1
(19)	*Metapenaeopsis dalei*	17	(49)	*Palaemon macrodactylus*	1
(20)	*Paradromia japonica*	17	(50)	Hermit-crab sp. 1	1
(21)	*Scyra compressipes*	16	(51)	*Nursia elegans japonicus*	1
(22)	Amphipoda sp.	13	(52)	*Leucosia longifrons*	1
(23)	*Plesionika* sp.	13	(53)	*Micippa thalia*	1
(24)	*Clibanarius bimaculatus*	8	(54)	*Achaeus stenorhynchus*	1
(25)	*Pinnotheres cardii*	8	(55)	*Pugettia nipponensis*	1
(26)	*Galathea orientalis*	7	(56)	*Pugettia* sp.	1
(27)	*Eupagurus trigonocheirus*	7	(57)	*Homoioplax haswelli*	1
(28)	*Cancer pygmaeus*	6	(58)	*Asthenognat. inaequipes*	1
(29)	*Pinnixa rathbuni*	5	(59)	*Hemigrapsus penicillatus*	1
(30)	*Pugettia quadridens*	4	(60)	Brachyura sp. 2	1

Latreutes planirostris, shrimps belonging to Hippolytidae, are found densely in the area-B only.

(7) Distribution of some important species

Under this heading, some notes will be made on distributions of some important species, namely *Crago affinis*, *Heptacarpus minutus*, *H. rectirostris*, *Crangon japonicus*, *Pilumnoplax vestita*, *Charybdis bimaculata*, *Trachypenaeus curvirostris*, and *Squilla oratoria*. Among those, *C. affinis*, *T. curvirostris*, and *S. oratoria* are available for human food. Therefore these species make elements important for shallow water fisheries in certain localities of Japan.

(a) *Crago affinis* (De HAAN)

Crago affinis is a common food shrimp inhabiting shallow waters. Of this

species of Tokyo Bay, total number of individuals and total weight in g per unit area in each station are shown graphically in Fig. 7.

This species is found to spread over all bottom area of this bay, but some seasonal dynamics of its horizontal distribution is seen. Considerably dense distribution is found to exist in the area-B and the east side of the bay at the season of late spring. But, in late summer season, the distribution density becomes evenly thin throughout all areas except station 80, in which a dense distribution is still kept (Fig. 7). At late autumn, the distribution becomes again densely in whole areas, and thicker in the east side of the bay than in the other side (Fig. 7, C, F). Remarkably enough, no distribution is found at station 15 throughout all samplings, and no specimens were obtained in the stations 5, 6, 15, 17, and 28 at the second sampling which is carried out in late summer season. Such scarcity of animal association seems to the present authors to be greatly caused by sea-water pollution due to drainage of cities, as the stations set forth above are locating near Tokyo and other cities.

Of this matter, some discussions will be given later on in relation to oceanographical conditions of such area.

(b) *Heptacarpus minutus* (Yokoya) and *H. rectirostris* (Stimpson)

These two species were found abundantly in the area-B (Fig. 1), especially in east half of the area (Stations 63, 65, 73) (Tables 12–14). A few of them were taken from southern part of the area-A (Stations 49 and 51) and some stations of the area-C (Stations 83, 84, 86, 87, and 88). No remarkable seasonal change was recognized in their distribution.

(c) *Crangon japonicus* (Miers)

A snapping shrimp, this is, inhabiting shallow waters. In Tokyo Bay, a rather salient seasonal variation of its distribution is found to exist. This one chiefly has been collected in the area-B and southern half of the area-A. It occurs most abundantly in late spring (Fig. 8 A, D), and least in late summer (Fig. 8 B, E). In late autumn, it appears more plentifully than in late summer. In the area-C, only one specimen was procured in the station 92 at the second sampling.

(d) *Pilumnoplax vestita* (De Haan)

This crab mostly inhabits bottoms deeper than 10 m (Fig. 9, A–F). In late spring land late summer seasons, it has considerably extensive distribution (Fig. 9, A, B, D, E), but the distribution in the latter season seems to be somewhat contracted in comparison to that in the late spring. It is of interest to note that the distribution appears to be thicker in the west side than in the east one in the late spring (Fig. 9, A, D). This trend is seen more sharply defined in the case dealt with gravimetric data of the same season. But the crab becomes suddenly very few in late autumn (Fig. 9 C, F). Only several specimens taken from four stations: 37, 67, 69, and 75.

Table 12. Number of individuals of each

Species \ Station	1	3	5	6	9	11	13	15	17	18	20	22	24	26	28	29	31	33	35	37	39	41
Squilla oratoria		1			3	7			1			1	2	1	1			1				
Trachypenaeusc urvirostris									1												1	
Metapenaeopsis acclivis																						
Metapenaeopsis dalei																						
Eusicyonia cristata																						
Plesionika sp.																						
Palaemon macrodactylus																						
Crangon bis-incisus																						
Crangon rapax																						
Crangon japonicus														3	2	1	3	4	1	3	2	2
Heptacarpus rectirostris																						
Latreutes planirostris																1						
Heptacarpus minutus																						
Spirontocaris pectinifera																						
Birulia kishinouyei																						
Leptochela aculeocaudata																						
Crago affinis	1		1	2		1			3	40	3			2	4	104	4	11	7	3	8	4
Sclerocrangon intermedius																						
Galathea elegans																						
Galathea orientalis																						
Porcellanera picta																			2			1
Clibanarius bimaculatus																						
Paguristes digitalis																						
Eupagrus constans																						
Eupagrus trigonocheirus																						
Paradromia japonica																						
Latreillia phalangium																						
Dorippe granulata																						
Nursia elegans																						
Arcania undecimspinosa	2	1		1																		
Leucosia longifrons																						
Achaeus japonicus																						
Pugettia qiadridens																						
Pugettia incisa																						
Pugettia minor																						
Pugettia nipponensis																						
Pugettia sp.																						
Scyra compressipes																						
Hyastenus diacanthus																						
Hyastenus elongatus																						
Pisoides ortmanni																						
Chorilia longipes															3							
Micippa thalia																						
Lambrus validus																						
Parthenopoides pteromerus																						
Lissocarcinus laevis																						
Charybdis bimaculata				1					2	1		1			5	1	2	3	1			2
Neptunus hastatoides																						
Kraussia integra																						
Cancer gibbosulus																						
Cancer pygmaeus																						
Actaea savignyi																						
Pilumnus minutus																						
Actumnus squamosus																						
Pilumnoplax vestita						1			1					1	1			1	10	8	7	2
Pinnotheres cardii						1										1						
Pinnixa rathbuni				1																		
Astenognathus inaequipes																						
Brachyura sp. 1																						
Brachyura sp. 2																						
Total	3	2	1	5	3	10			8	41	3	2	3	10	13	107	10	29	19	13	11	11

species by stations at the first sampling.

43	45	47	49	51	53	55	57	59	61	63	65	67	69	71	73	75	77	80	81	82	83	84	85	86	87	88	89	90	91	92	Total
	4				3						1					3	3		2	1	1										36
					1			3	4		2	30	1		14	6		6	3	20											92
							2																						1		3
												6								5		4		3		8			6	32
								2							7				1							1					1
																	1													10
																														1
																												1		1
	4		23	4	5	4	4	7	3	1	2	30	10	10	6	20	6	2	21	33						1				1
										67	16	2			2					2										216
										10	1	1			9					2	1	5				1				89
			1							14	559				1					37					1					31
										1	12	2			1					1										613
										1	1	8			2	1				6										17
															1															19
24			143		7	1	4	91	10		15	414	6	3	58	116	14	263	49	337		1								1
												4			3					5										1756
																									6					12
																				2						14				6
						1			6							5		1		1											16
											1	1														2					17
										1		2								17	1										2
																				7					1						21
										3		1								2											8
												1														4					6
												1								1											4
											3	2			3					2						8					2
										1																					9
																										2					14
											1																				1
										5																					2
												1														3					1
											1																				5
										2	1	2								5						1					3
																									2	1					1
																								1		2					3
																				1											3
																					1										1
												1																			1
2		15		5		1	10	5		2	28			18	21		7	3	17		1					1					1
																				4											154
												1														1					4
												1								1											1
										1	1									2											2
												2								10					6	4	2				2
11		2	4	34	34	2	2	9			2	31	10		3	35	1	25	10				1		2						24
																															5
														1																	247
																				1											2
																									1						2
																										5					1
																															1
45		184	8	55	40	13	115	37	106	619	544	48	23	126	175	59	280	104	528	8	11		4	1	19	60	3	2	6	3528

Table 13. Number of individuals of each

Species \ Station	1	3	5	6	9	11	13	15	17	18	20	22	24	26	28	29	31	33	35	37
Squilla oratoria				1	1	1	5				3		3				1	2		2
Amphipoda sp.																				
Cirolana japonensi																				
Cleantis planicauda																				
Idotea metallica																				
Trachypenaesus curuirostris																				
Metapenaeopsis dalei																				
Plesionika sp.																				
Crangon japonicus																	1		1	
Heptacarpus rectirostris																				
Latreutes planirostris																				
Heptacarpus minutus																				
Spirontocaris pectinifera																				
Birulia kishinouyei																				
Leptochela aculeocaudata																				
Leptochela gracilis																				
Crago affinis	21	4			1	6	6						2	5		4	18			
Sclerocrangon intermedius																				
Galathea orientalis																				
Porcellanella picta																				
Diogenes edwardsii																				
Eupagurus constans																				
Hermit-crab sp.																				
Paradromia japonica																				
Dorippe granulata																				
Arcania undecimspinosa	1						3													
Philyra platycheira																				
Achaeus tuberculatus																				
Pugettia quadridens																				
Pugettia incisa																				
Pugettia minor																				
Scyra compressipes																				
Micippa thalia																				
Charybdis japonica																				
Charybdis bimaculata																1	1		1	
Neptunus sanguinolentus																				
Neptunus gladiator																				
Neptunus hastatoides																				
Thalamita oculea																				
Cancer pygmaeus																				
Xanthias elegans																				
Actaea savignyi																				
Pilumnus minutus																				
Pilumnoplax vestita														1	4				1	5	3
Pinnotheres cardii	1											1								
Total	23	4		1	2	10	11				4		6	9		5	21	3	7	5

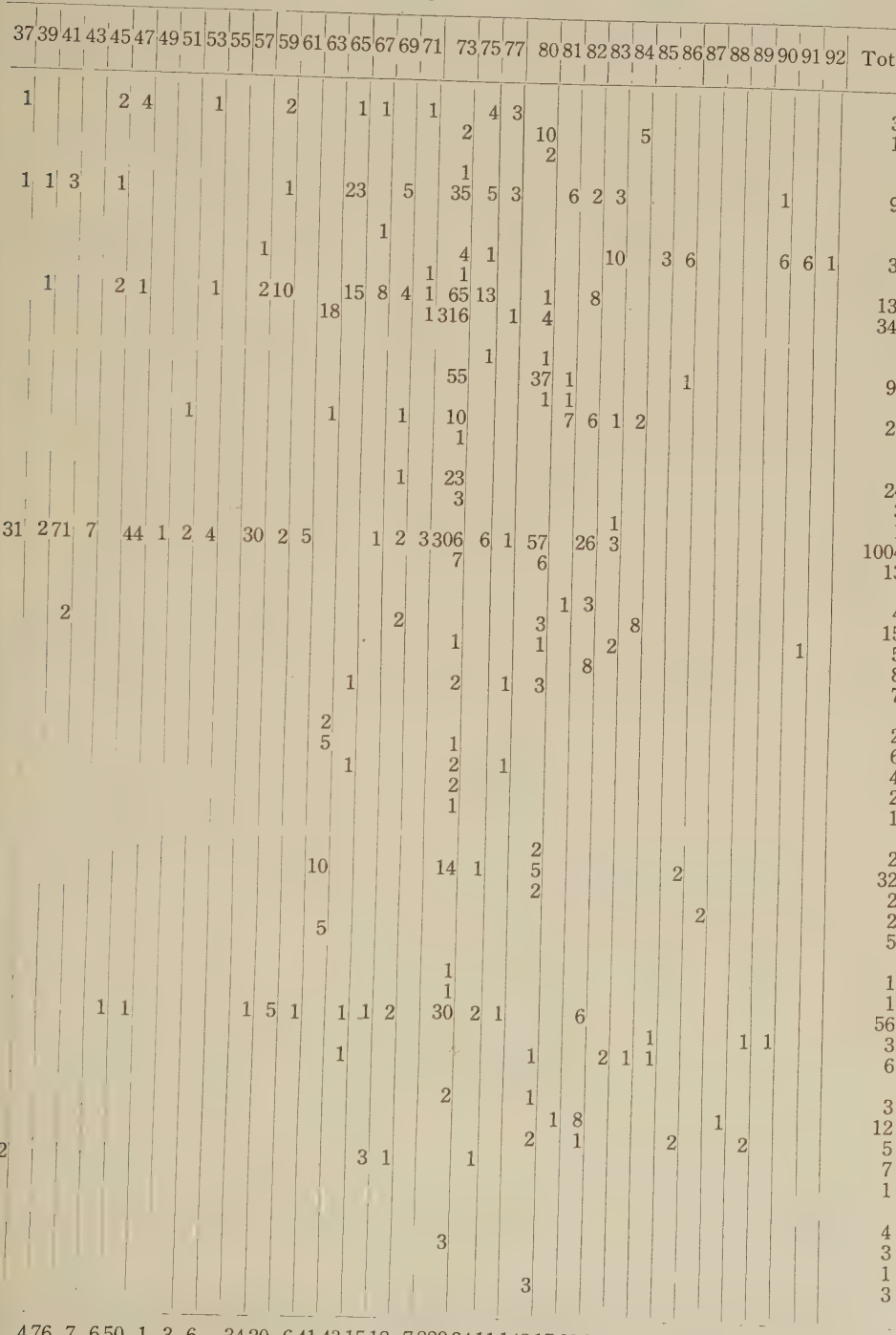
species by stations at the second sampling.

39	41	43	45	47	49	51	53	55	57	59	61	63	65	67	69	71	73	75	77	80	81	82	83	84	85	86	87	88	89	90	91	92	*	Total
	1	1	2					2		1						3			3	1	4											2	4	43
													1																			3	4
													1																		1		2
																															5		5
																															8		8
																1				1	2									1	46		51
																												8					8
1							1	2		1	4	1		7		5			3		1												1
												1	45				2	1			6										1		34
																					1				1							130	181
	2																	1															8
												38	93			15		1			8				4	26				1			214
													15				1															33	20
													1																			4	3
																		1														2	1
																1																	1
	7	4	1	2	1		1	1	1	3			2		9		11	10	84	6											3	3	216
																	1																1
								1		1			1	2												1		1					3
																																	4
											1	1					1		1	8	1								1		2		3
																					1												13
											1	3								1											1		2
																															1		5
																				1													1
																															1		6
																1															1		1
											1												1										1
												1				1					2				1				12	1			2
																																	18
																				1								3					3
																													1				1
1	3	2		3	1		1	4	4		3	1	2	3		10		2		6											3		3
																															1		50
																															5		5
																								1									1
																													1	10			11
																				1									1				1
																																	1
																					1								1				1
											1				10				4	10													1
2	5	7	8		2	2	4	6		3	8		1		7			6		10								1					26
																																	85
																																	2
4	18	14	11	5	4	2	7	16	5	9	15	45	166	13		35	31	19	22	87	60	15		1	1	6	27		15		16	76	196	1052

Table 14. Number of individuals of each

Species \ Station	1	3	5	6	9	11	13	15	17	18	20	22	24	26	28	29	31	33	35
Squilla oratoria		1		4	2		1		2				1	1	1				
Amphipoda sp.																			
Idotea sp.																			
Isopoda sp.																			
Trachypenaeus curvirostris									3				1		1			1	1
Metapenaeopsis acclivis																			
Metapenaeopsis dalei																			
Plesionika sp.																			
Crangon japonicus														1				2	1
Heptacarpus rectirostris																			
Palaemon serrifer																			
Latreutes planirostris																			
Lysmata vittata																			
Heptacarpus minutus																			
Spirontocaris pectinifera																			
Leptochela aculeocaudata																			
Leptochela gracilis																			
Processa japonica																			
Crago affinis		38		7	19	40	1		3	65	79	2	44	36	13	20	1	25	7
Sclerocrangon intermedius																			
Galathea orientalis																			
Porcellanella picta																			
Diogenes edwardsii																			
Clibanarius bimaculatus																			
Eupagrus constans																			
Hermit-crab sp.																			
Paradromia japonica																			
Arcania undecimspinosa																			
Achaeus stenorhynchus																			
Pugettia quadridens																			
Pugettia incisa																			
Pugettia minor																			
Hyastenus diacanthus																			
Scyra compressipes																			
Pisoides ortmanni																			
Parthenopoides pteromerus																			
Charybdis bimaculata									1								1	1	1
Neptunus gladiator																			
Neptunus hastatoides																			
Cancer pygmaeus																			
Pilumnus minutus																			
Actumnus squamosus																			
Pilumnoplax vestita																			
Homoioplax haswelli																		1	
Pinnotheres cardii							3						1						
Pinnixa rathbuni																			
Hemigrapsus penicillatus															1				
Brachyura sp. 1																			
Total		39		11	21	40	5		9	65	79	2	47	38	16	20	2	30	10

species by stations at the third sampling.



37	39	41	43	45	47	49	51	53	55	57	59	61	63	65	67	69	71	73	75	77	80	81	82	83	84	85	86	87	88	89	90	91	92	Total
1				2	4			1			2			1	1		1		4	3															33
																		2			10				5									17
																					2													2
1	1	3		1							1		23		5			1																1
																		35	5	3		6	2	3							1			97
															1																			1
										1								4	1					10		3	6				6	6	1	38
	1			2	1			1		2	10			15	8	4	1	1																2
													18				1	65	13		1		8											136
																	1	316		1	4													340
																			1		1													
																		55			37	1				1								2
																					1	1												94
							1						1			1		10				7	6	1	2									2
																		1																29
																1		23																1
																		3																24
31	2	71	7	44	1	2	4		30	2	5			1	2	3	3	06	6	1	57		26	1 3										3 1
																		7			6			3										1004
																																		13
		2													2						3	1	3											4
																		1			1			2	8						1			15
																							8											5
													1					2		1	3													8
													2																					7
													5					1																2
														1				2		1														6
																		2																4
																		1																2
																					2													2
												10						14	1		5					2								32
																					2						2							2
												5																						2
																																		5
																	1																	1
																	1																	1
			1	1					1	5	1		1	1	2		30	2	1			6			1				1	1				56
													1								1		2	1	1										3
																	2				1														6
																						1	8					1							3
2														3	1				1		2		1			2			2						12 5
																																		7
																																		1
																	3																	4
																																		3
																				3														1
																																		3
35	4	76	7	6	50	1	3	6		34	20	6	41	43	15	18	7	889	34	11	142	17	68	22	16	5	11	2	1	3	8	7	1	2043

Table 15. Number of species, number of individuals, and weight in g in each station at each sampling. *, station unknown. Av., average; FS, first sampling; F–T, first-third sampling combined; SS, second sampling; St., station; TS, third sampling.

Items / St.	Number of species				Number of individuals				Weight			
	FS	SS	TS	T–S	FS	SS	TS	Total	FS	SS	TS	Total
1	2	3		3	3	23		26	9.1	6.1		15.2
3	2	1	2	3	2	4	39	45	4.5	0.3	15.9	20.7
5	1			1	1			1	0.5			0.5
6	4	1	2	5	5	1	11	17	15.7	22.2	41.6	79.5
9	1	2	2	2	3	2	21	26	18.0	13.3	12.8	44.1
11	4	3	1	5	10	10	40	60	26.2	28.0	9.0	63.2
13		2	3	3		11	5	16		86.0	24.4	110.4
15												
17	5		4	5	8		9	17	24.3		26.5	50.8
18	2		1	2	41		65	106	20.3		21.3	41.6
20	1	2	1	3	3	4	79	86	2.0	89.7	12.9	104.6
22	2		1	3	2		2	4	16.2		0.6	16.8
24	2	3	4	5	3	6	47	56	24.8	70.0	54.1	148.9
26	5	2	3	5	10	9	38	57	19.3	29.6	28.2	77.1
28	5		4	7	13		16	29	94.6		15.4	110.0
29	4	2	1	4	108	5	20	133	32.2	6.4	4.0	42.6
31	4	4	2	5	10	21	2	33	23.3	40.0	1.9	65.2
33	5	2	5	7	29	3	30	62	87.9	23.5	19.8	131.2
35	5	3	4	6	19	7	10	36	38.2	34.6	12.7	85.5
37	3	2	4	5	13	5	35	53	31.5	58.4	53.9	143.8
39	3	3	3	5	11	4	4	19	10.7	30.8	5.0	46.5
41	5	5	3	8	11	18	76	105	23.0	123.0	22.7	168.7
43		4	1	4		14	7	21		71.6	2.3	73.9
45	5	3	4	6	45	11	6	62	125.9	62.5	7.6	196.0
47		2	4	4		5	50	55		23.9	69.0	92.9
49	5	3	1	5	184	4	1	189	187.4	24.7	0.3	212.4
51	2	1	2	4	8	2	3	13	11.2	6.5	1.3	19.0
53	6	4	3	6	55	7	6	68	235.7	12.7	7.1	255.5
55	4	6		6	40	16		56	96.5	77.6		174.1
57	5	2	4	6	13	5	34	52	23.8	32.8	11.7	68.3
59	6	5	5	8	115	9	20	144	126.7	37.4	20.4	184.5
61	6	3	2	6	37	15	6	58	80.8	53.8	1.9	136.5
63	11	8	6	15	106	45	41	192	61.5	24.4	67.8	153.7
65	16	12	7	23	619	166	43	828	135.3	32.5	52.0	219.8
67	24	4	6	27	544	13	15	572	522.2	31.4	21.9	575.5
69	4		8	8	48		18	66	73.1		12.3	85.4
71	3	6	5	8	23	35	7	65	34.0	163.9	2.7	200.6
73	14	7	26	28	126	31	889	1046	111.9	5.3	197.7	314.9
75	8	8	9	17	175	19	34	228	257.9	3.8	18.1	279.8
77	5	4	7	10	59	22	11	92	152.0	115.6	20.1	287.7
80	6	4	19	23	280	87	142	509	136.4	34.4	29.6	200.4
81	7	15	6	18	104	60	17	181	128.4	220.4	14.6	363.4
82	26	5	9	30	528	15	68	611	424.6	16.8	28.7	470.1
83	5		7	11	8		22	30	31.2		47.4	78.6
84	4	1	4	9	11	1	16	28	10.6	4.0	1.8	16.4
85		1	3	3		1	5	6		30.0	16.4	46.4
86	2	3	4	5	4	6	11	21	9.0	0.7	3.1	12.8
87	1	2	1	4	1	27	2	30	0.5	1.8	3.8	6.1
88	7		1	7	19		1	20	6.1	0.1	1.8	8.0
89	19	6	2	22	60	15	3	78	21.8	15.9	6.8	44.5
90	2		3	5	3		8	11	4.3		9.5	13.8
91	2	5	2	9	2	16	7	25	5.0	4.2	7.1	16.3
92	1	17	1	18	6	76	1	83	18.5	243.5	1.4	263.4
*		8		8		196		196		95.3		95.3
Total	271	189	212	672	3528	1052	2043	6623	3561.5	2109.5	1068.4	6739.4
Av.	5.1	3.6	4.0	4.22	66.56	19.85	38.54	41.65	67.14	39.76	20.20	42.37

Table 16. Weight in g of each species by stations at the first sa

Species \ Station	1	3	5	6	9	11	13	15	17	19	20	22	24	26	28	29	31	33	35	37	39	41	43	45	47	49
illa oratoria		2.2			18.0	22.2			6.0			14.9	15.9	6.1	58.6			32.6						24.0		
achypenaeus curvirostris									7.1												3.0					
tapeneopsis acclivis																										
tapeneopsis dalei																										
sicyonia cristata																										
sionika sp.																										
laemon macrodactylus																										
angon tis-incisus																										
angon rapax																										
angon japonicus														4.8	5.1	0.5	5.9	4.8	1.2	5.2	3.8	2.6		6.1		37.
ptacarpus rectirostris																										
ptacarpus minutus																										
treutes planirostris																0.2										
irontocaris pectinifera																										0.
ulia kishinouyei																										
otochela aculeocaudata																										
ago affinis	0.6		0.5	1.7		1.2			3.3	16.8	2.0			1.7	4.0	28.1	4.1	9.2	5.1	3.4	3.9	3.3		19.1		83.
erocrangon intermedius																										
lathea elegans																										
lathea orientalis																										
rcellanella picta																			1.6			0.4				
banarius bimaculatus																										
guristes digitalis																										
pagrus constans																										
pagrus trigonocheirus																										
radromia japonica																										
treilla phalangium																										
rippe granulata																										
rsia elegans																										
cania undecimspinosa	8.5	2.3		7.8																						
ucosia longifrons																										
haeus japonicus																										
gettia quadridens																										
gettia incisa																										
gettia minor																										
gettia nipponensis																										
gettia sp.																										
ra compressipes																										
astenus diacanthus																										
astenus elongatus																										
oides ortmanni																										
rilia longipes														4.4												
ippa thalia																										
nbrus validus																										
rthenopoides pteromerus																										
socarcinus laevis																										
rybdis bimaculata				2.6					2.8	3.5		1.3			26.0	3.4	5.6	13.1	1.6			9.8		8.9		64
otunus hastatoides																										
ussia integra																										
cer gibbosulus																										
cer pygmaeus																										
ae a savignyi																										
u inus minutus																										
u inus squamosus																										
u noplax vestita						1.8			5.1				8.9	2.3			7.7	28.2	28.7	22.9		6.9		67.8		2.
n theres cardii						1.0									0 9											
nixa rathbuni				0.5																						
penognathus inaequipes																										
hyura sp. 1																										
hyura sp. 2																										

ling. Total of each station see the Table 18.

51	53	55	57	59	61	63	65	67	69	71	73	75	77	80	81	82	83	84	85	86	87	88	89	90	91	2	Total
	66.2						1.7					64.4	73.2		12.5	2.8	4.0										425.3
	5.2			9.9	20.7		3.7	56.0	3.5		22.2	15.2		8.7	5.5	24.0									3.6		184.7
			6.6					16.9								11.5		8.3		7.1			12.9			18.5	10.2
																							0.9				75.2
				1.8							0.5				1.0												0.9
													1.1														3.3
																								4.1			1.1
																							0.2				4.1
6.1	10.4	5.9	5.3	11.3	4.3	0.9	2.1	37.3	14.6	14.6	6.4	24.7	7.8	1.1	13.8	41.4											0.2
						3.95	2.1	1.4			1.0					0.7											285.5
						1.1	67.6				0.1					6.2						0.1					9.15
						2.4	0.5	0.2			1.5					0.5	0.3	1.4					0.4				75.2
						0.1	1.6	0.4			0.1					0.2											7.4
						0.6	0.8	7.2			1.6	0.6				2.9											2.4
											0.1																13.7
	7.6	0.4	3.0	53.1	7.6		8.1	236.3	4.1	3.3	17.2	54.6	9.6	103.1	35.2	175.9		0.6									0.1
								2.9			1.6					3.2									1.4		912.4
																						0.7					7.7
																0.3							0.9				0.7
		0.5			2.5							2.2		0.7		0.2											1.2
																							0.7				8.1
							16.3	3.8																			0.7
						0.6		2.6								21.1	1.9										20.1
																9.7						0.1					26.2
						9.3		1.3								7.5											9.8
																							0.9				18.1
								6.1								3.5											0.9
								0.05															0.2				9.6
							2.7	6.0			3.6					7.2											0.25
						6.9																					38.1
																							0.3				6.9
							1.9																				0.3
						30.4																					1.9
																							0.1				30.4
								0.1																			0.1
							3.0																				0.1
						5.3	15.5	3.0								13.9											3.0
																							1.9				37.7
																						1.8	0.1				1.9
																					0.5		0.1				1.9
																											0.6
																13.3											4.4
																	15.7										13.3
								0.6																			15.7
																							0.5				0.6
	23.8		3.5	47.2	18.6		7.3	140.5			55.9	81.1		20.3	18.6	53.5		0.3									0.5
																	9.3										613.4
																							0.1				9.3
								0.5															0.3				0.1
								0.7								0.2											0.8
																4.8											0.9
							0.4	0.2								6.2						1.3	1.1	0.2			4.8
								0.5												1.9		2.0					9.4
5.1	122.5	89.7	5.4	3.4	27.1			4.7	50.9	16.1		15.1	60.3	2.5	41.8	13.7											4.4
																											640.9
											0.1																1.9
																0.2											0.6
																											0.2
																						0.1					0.1
																							0.2				0.2

Table 17. Weight in g of each species by stations at the second sampling.

Species \ Station	1	3	5	6	9	11	13	15	17	18	20	22	24	26	28	29	31	33	35	37	39	41	43	45	47	49
illa oratoria				22.2	12.8	13.7	85.5				89.2		57.7				26.6	22.5		27.2		60.6	14.2	36.5		
phipoda sp.																										
olana japonicus																										
antis planicauda																										
tea metallica																										
chypenaeus curvirostris																										
tapenaeopsis dalei																										
sionika sp.																										
ngon japonicus																	2.0		3.5		2.1					
otacarpus rectirostris																										
reutes planirostris																										
otacarpus minutus																						0.4				
rontocaris pectinifera																										
ulia kishinouyei																										
otochela aculeocaudata																										
otochela gracilis																										
go affinis	2.1	0.3			0.5	0.9	0.5						0.8	0.5		0.4	2.5					0.9	1.0	0.7	0.7	0.5
erocrangon intermedius																										
athea orientalis																										
rcellanella picta																										
genes edwardsii																										
pagrus constans																										
mit-crab sp.																										
adromia japonica																										
ippe granulata																										
ania undecimspinosa	3.9					13.4																				
lyra platycheira																										
aeus tuberculatus																										
gettia quadridens																										
gettia incisa																										
gettia minor																										
ra compressipes																										
ippa thalia																										
rybdis japonica																										
rybdis bimaculata																6.0	8.9		8.1		6.3	22.1	17.1		23.2	7.0
otunus sanguinolentus																										
otunus gladiator																										
otunus hastatoides																										
llamita oculea																										
cer pygmaeus																										
othias elegans																										
ucea savignyi																										
mnus minutus																										
mnoplax vestita													11.8	29.1				1.0	23.0	31.2	21.9	39.0	39.3	25.3		17.2
notheles cardii	0.1										0.5															

station unknown. Total of each station see the Table 18.

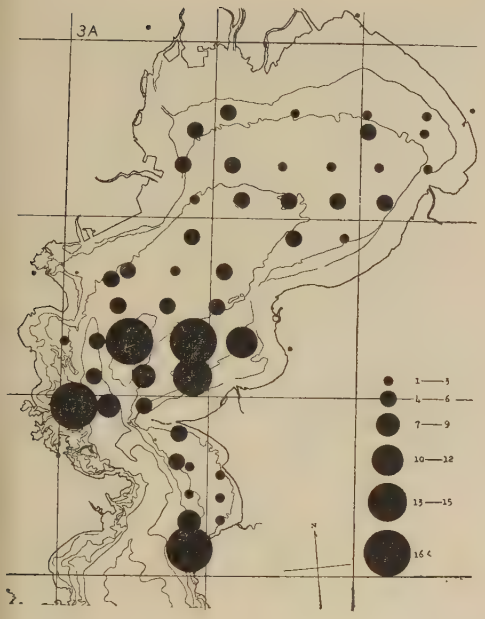
51	53	55	57	59	61	63	65	67	69	71	73	75	77	80	81	82	83	84	85	86	87	88	89	90	91	92	*	Total
		35.7		17.0						40.5			92.5	16.4	87.3											43.0	86.4	887.5
							0.1																				0.1	0.2
							0.1																			0.1		0.2
																										0.4		0.4
																										0.5		0.5
										10.8				1.1	10.2										0.9	47.0		70.0
																							11.1					11.1
															1.7													1.7
	1.8	2.5		2.3	5.5	1.5		7.5		8.7			4.6		6.8											3.7		52.5
						0.2	0.9				0.1	0.1			0.1					0.1							3.4	4.9
											0.1	0.1								0.2						0.1		0.8
						3.1	7.3				1.0	0.1			1.0						1.7						3.1	17.3
							0.9				0.1																0.3	1.3
							0.2																				0.3	0.5
												0.1																0.1
											0.1																	0.1
	0.2	0.1	0.1	0.5			0.2			3.7		1.3	2.5	14.4	2.1											0.7	0.5	34.3
												0.1																0.1
							0.1														0.1	0.1						0.3
		0.4		0.6				0.3																				1.3
						5.5	2.4					0.4		2.5	28.5	3.8							2.9			22.1		25.0
																0.5												43.1
						2.1	2.7								1.2												1.2	1.7
																												6.0
																										0.2		0.2
															8.7											6.0		32.0
											0.1															0.1		0.1
						2.6																						0.1
							0.1				0.3					4.7		4.0		0.4					2.1	0.9		6.6
																							0.3					8.2
															1.3													0.3
																							1.5					1.3
	6.5	25.2	32.7		19.7	9.2	20.2	23.2		65.2		1.6			30.6											83.7		1.5
																										6.5		83.7
																										21.0		339.3
																			30.0									21.0
																									0.8	7.7		30.0
															0.9										0.3			8.5
																												0.3
																0.1									0.1			0.9
						0.2					3.6				1.9	7.7							0.1					0.1
6.5	4.2	13.7		17.0	28.6			0.4		35.0			16.0		38.1													0.1
																												13.5
																												398.3
																												0.6

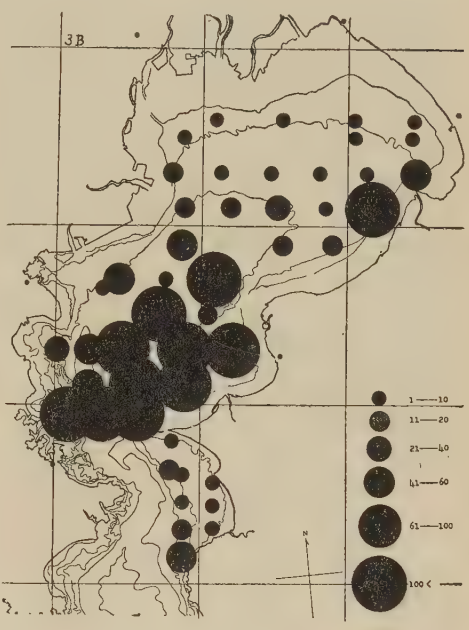
Table 18. Weight in g of each species by stations at the third

Species \ Station	1	3	5	6	9	11	13	15	17	18	20	22	24	26	28	29	31	33	35	37	39	41	43	45	47
lla oratoria		2.1		39.1	6.7		21.0		12.5				36.1	19.2	6.1					37.7				1.0	49.
hipoda sp.																									
oda sp.																									
ea sp.																									
chypenaeus curvirostris									9.5				4.2		3.3			2.2	7.1	5.9	4.2	6.1		3.0	
apenaeopsis acclivis																									
apenaeopsis dalei																									
ionika sp.																									
ngon japonicus														0.3				0.8	2.2		0.4			2.9	0.
tacarpus rectirostoris																									
aemon serrifer																									
reutes planirostris																									
nata vittata																									
tacarpus minutus																									
ontocaris pectinifera																									
ochela acculeocaudata																									
ochela gracilis																									
cessa japonica																									
po affinis		13.6		2.5	6.1	9.0	0.4		1.2	21.3	12.9	0.6	13.1	8.7	5.0	4.0	0.2	6.1	1.8	9.9	0.4	16.4	2.3		14.
rocrangon intermedius																									
athea orientalis																									
cellanella picta																						0.2			
enes edwardsii																									
anarius bimaculatus																									
agurus constans																									
nit-crab sp. 1																									
adromia japonica																									
unia undecimspinosa																									
aeus tuberculatus																									
aeus stenorhynchus																									
ettia quadridens																									
ettia incisa																									
ettia minor																									
stenus diacanthus																									
ra campressipes																									
ides ortmanni																									
thenopoides pteromerus									3.3								1.7	0.6	1.6					0.7	4
rybdis bimaculata																									
tunus gladiator																									
tunus hastatoides																									
cer pygmaeus																									
mnus minutus																									
mnus squamosus																					2.4				
mnoplax vestita																		10.1							
oplax haswelli							3.0						0.7												
heres cardii																									
heres rathbuni															1.0										
igrapsus penicillatus																									
hyura sp.																									

sampling. Total of each station see the Table 18.

49	51	53	55	57	59	61	63	65	67	69	71	73	75	77	80	81	82	83	84	85	86	87	88	89	90	91	92	Total
		3.8			3.6			1.2	2.2		0.6		3.2	10.5														255.6
												0.1			0.1				0.1									0.3
												0.1																0.1
															0.1													0.1
					3.6			35.7		5.6		21.5	1.7	2.8		9.5	4.8	9.3							1.7			141.7
									8.1																			8.1
				0.7								0.9	0.6					8.9		2.0	2.0				6.6	2.9	1.4	26.0
											0.2	0.02																0.22
		1.8		1.1	6.3			7.2	8.3	2.2	0.4	25.9	7.3		0.5		3.9											71.8
							1.3				0.3	42.1		0.1	0.6													44.4
													0.2		0.6													0.8
												1.63			2.4	0.1				0.04								4.17
																0.1												0.1
	0.5						0.2			0.4		2.0			0.4	2.0	1.4	0.3	0.6									7.8
												0.2																0.2
										0.1		0.8																0.9
												0.02																0.02
																		0.1										0.1
0.3	0.8	1.5		8.4	0.4	1.4			0.2	0.5	1.2	52.0	2.1	0.2	11.4		6.8	1.0										238.5
												1.4			1.6													3.0
																0.1	0.2											0.3
										1.3					0.6				0.9									3.0
												2.4			2.3			24.1								4.2		33.0
																	1.1											1.1
								5.2				8.0		2.3	4.3													19.8
							2.9																					2.9
							14.4					6.6																21.0
								0.5				0.8		3.8														5.1
												0.5																0.5
												0.1																0.1
							13.8					11.7	0.9			1.5					0.6							28.5
																0.7												0.7
																						3.8						3.8
							35.2																					35.2
												0.2																0.2
												0.05																0.05
9				1.5	6.5	0.5		0.5	0.3	1.1		18.1	1.1	0.4			4.0			14.0				3.8	1.2			46.8
								1.7							1.1			3.7	0.2	0.4								19.0
																												7.1
												0.5			0.7													1.2
															1.2	0.6	6.2						1.8					9.8
																	0.3				0.5			3.0				3.8
									2.8	1.1																		7.3
																												10.1
																												3.7
												0.1																0.1
																												1.0
															0.2													0.2
															1.5													1.5





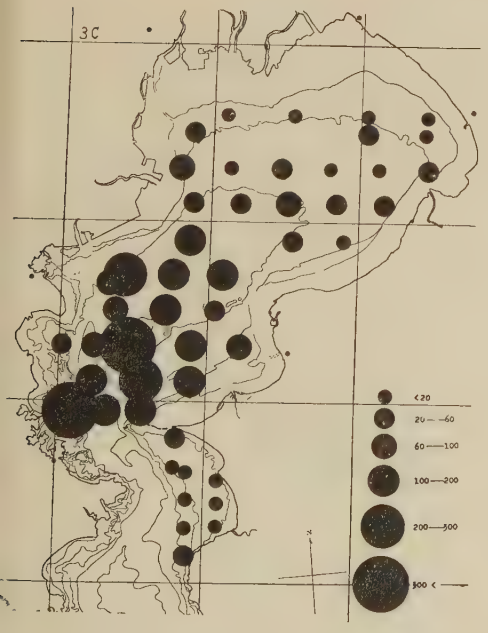


Fig. 3. Distribution of number of species (A), number of individuals (B), and weight in g (C) at the first sampling.

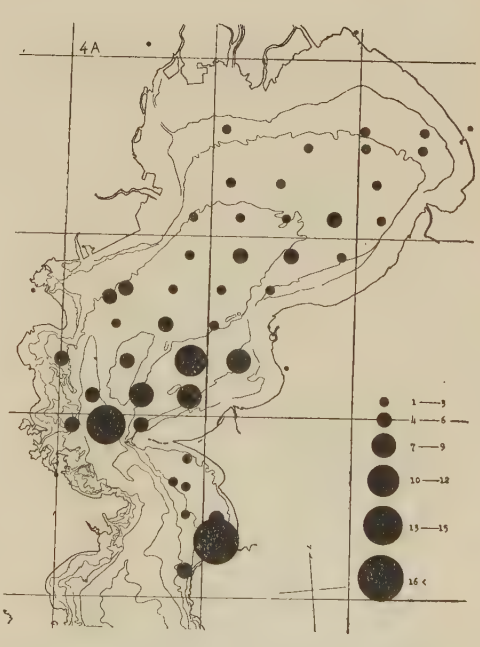
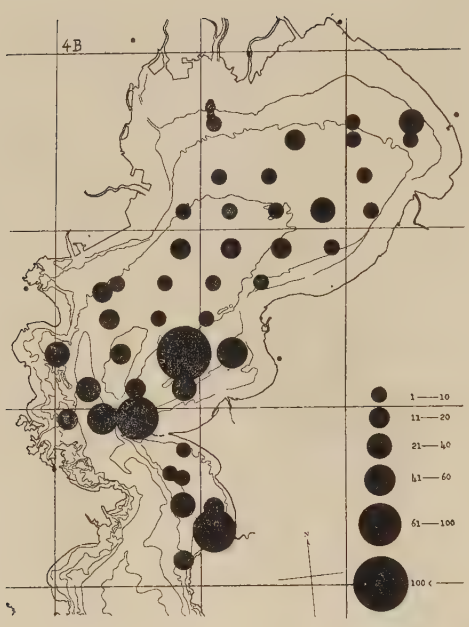
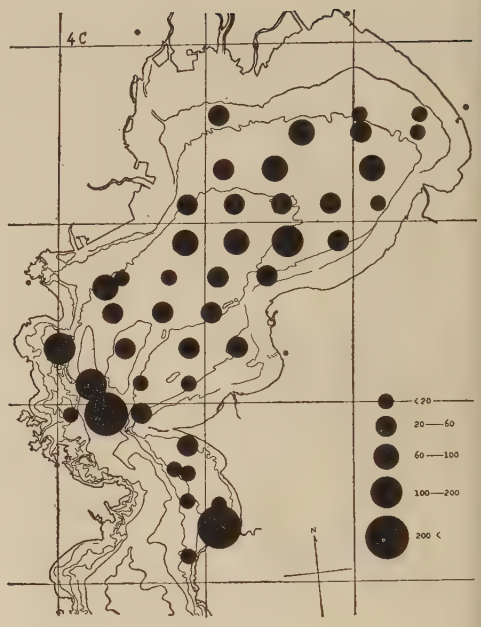
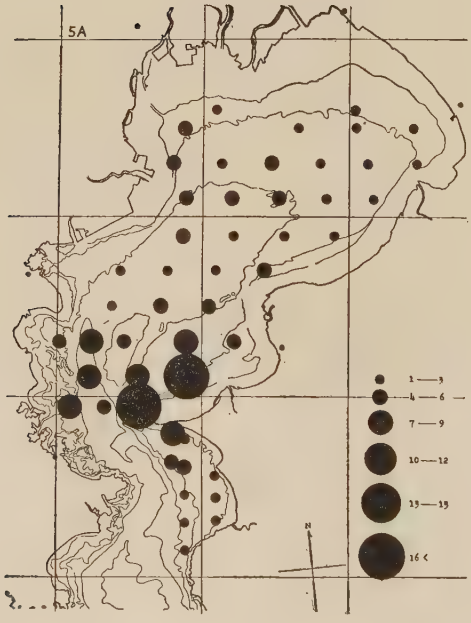


Fig. 4. The same as in the Fig. 3, at the second sampling.







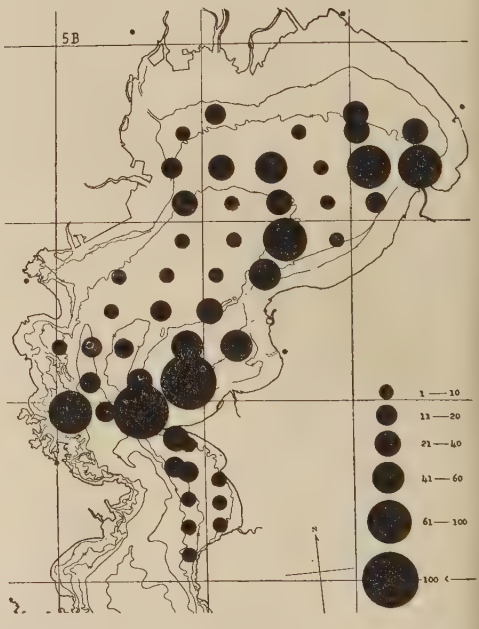


Fig. 5. The same as in the Fig. 3, at the third sampling.

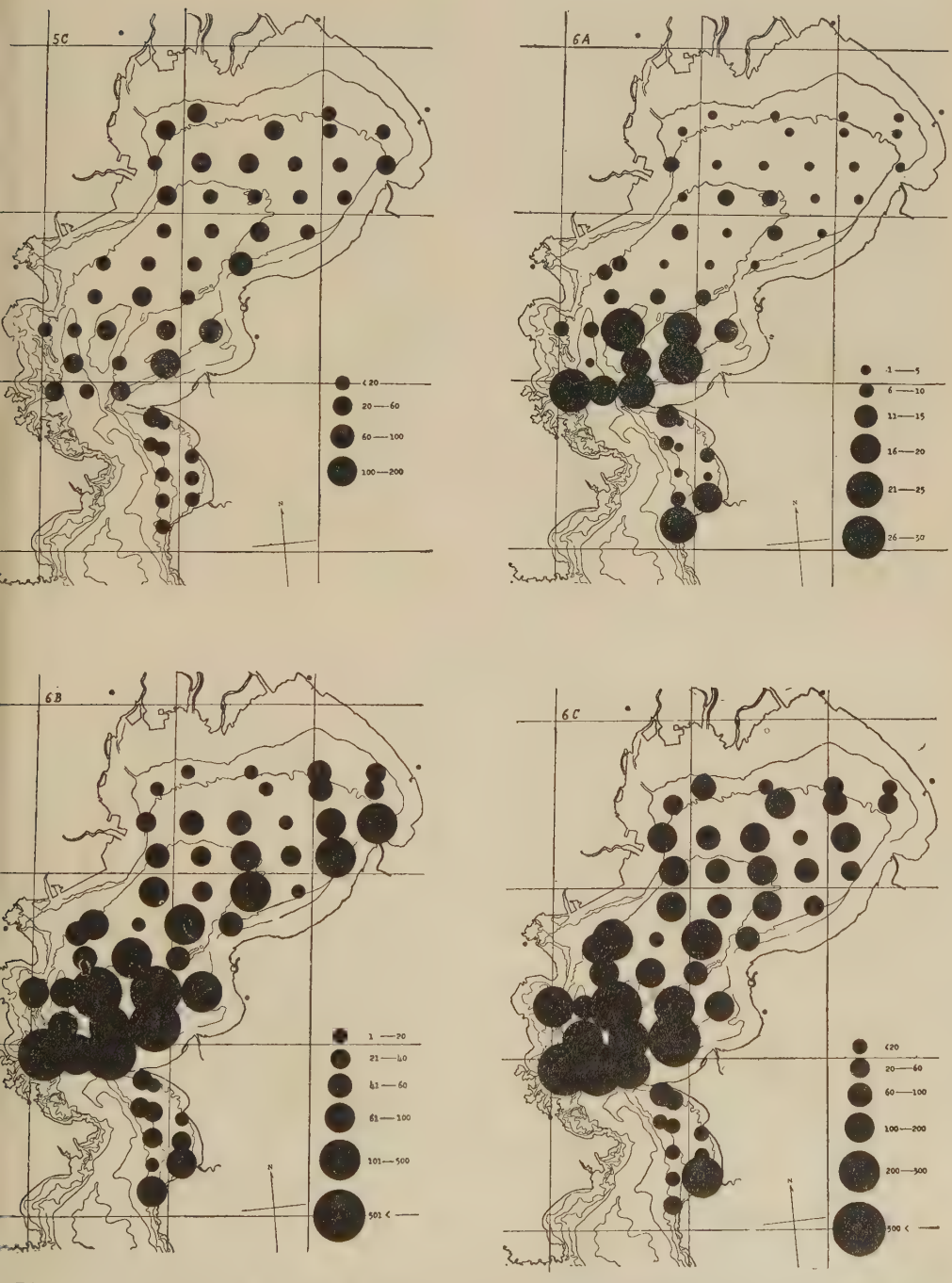


Fig. 6. Distribution of number of species (A), number of individuals (B), and weight in g (C) lt with all the material combined those obtained in all the three samplings from the first the third.

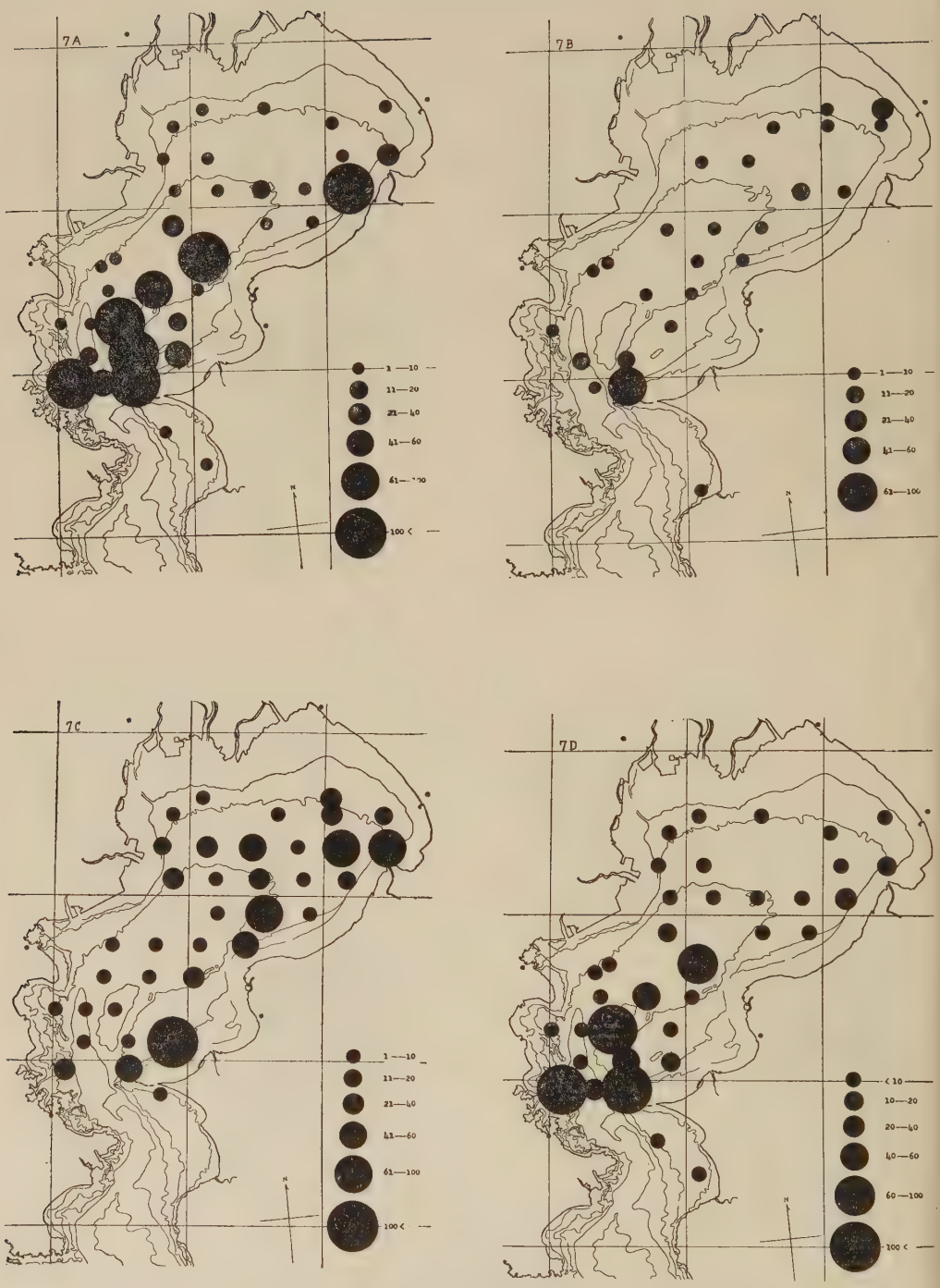


Fig. 7. Distribution of number of individuals (A–D) and weight (D–F) par unit area of the first (A, D), second (B, E), and third (C, F) samplings of *Crago affinis*.

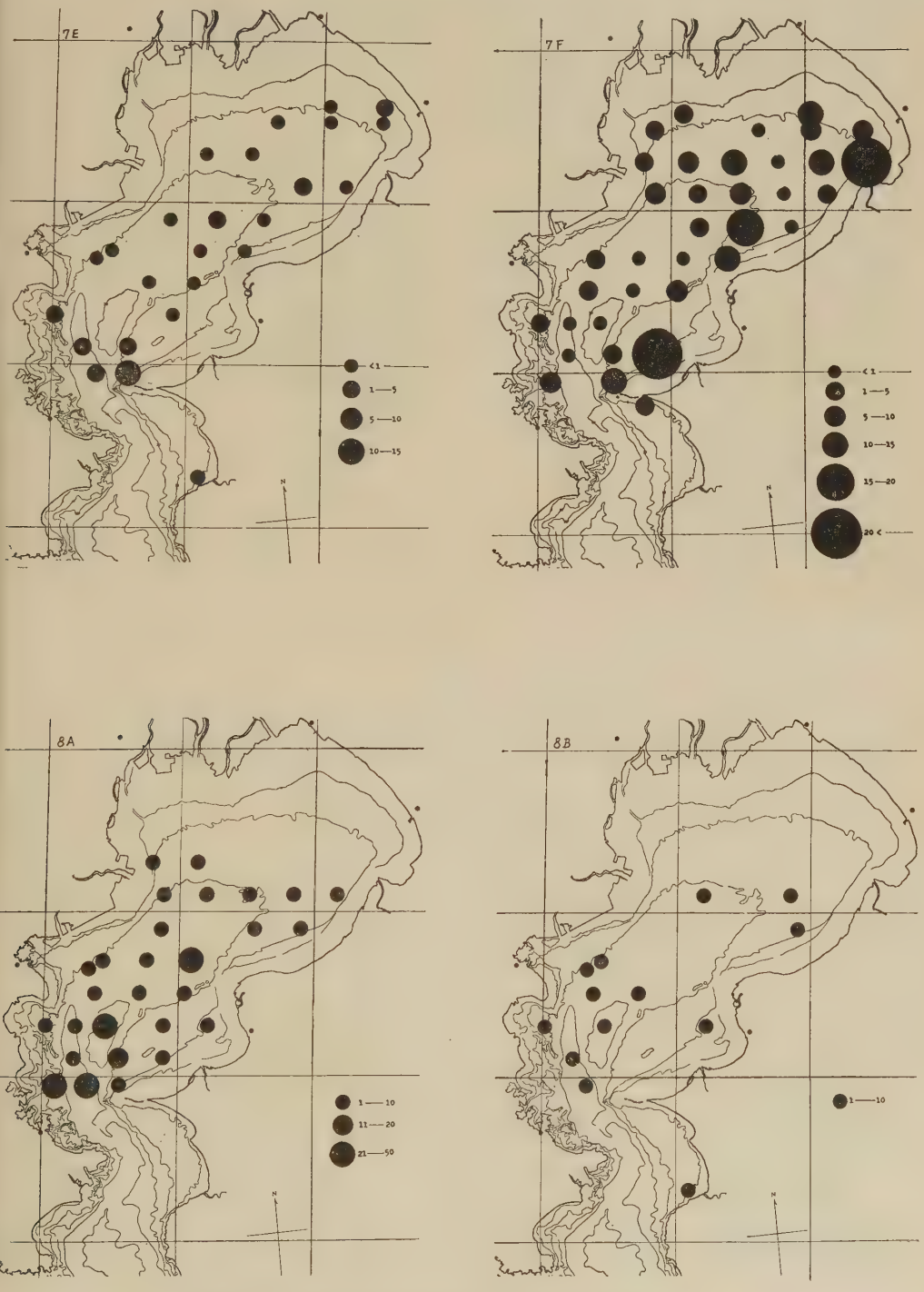
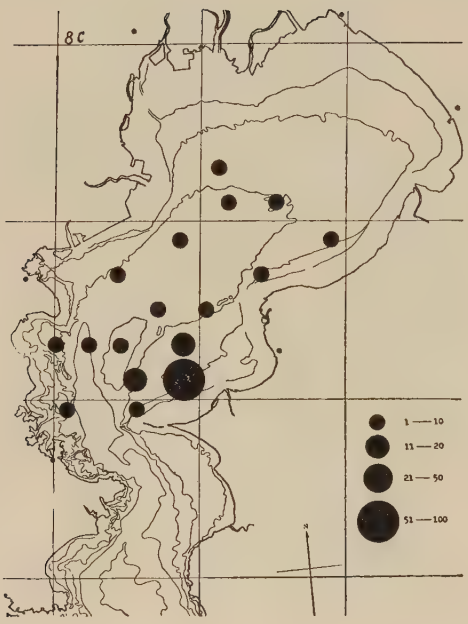
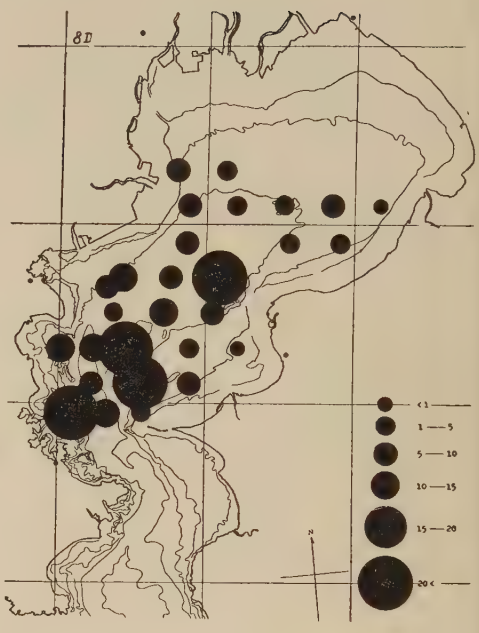
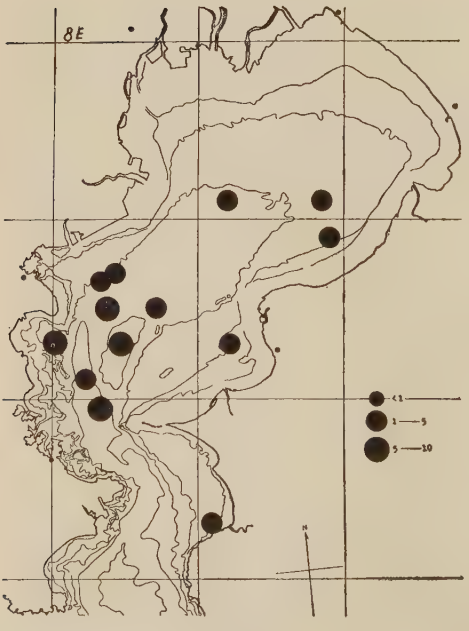


Fig. 8. *Crangon japonicus*. For A–F, see Fig. 7.


8C
1 — 10
11 — 20
21 — 50
51 — 100
N


8D
< 1
1 — 5
5 — 10
10 — 15
15 — 20
20 <
N


8E
< 1
1 — 5
5 — 10
N

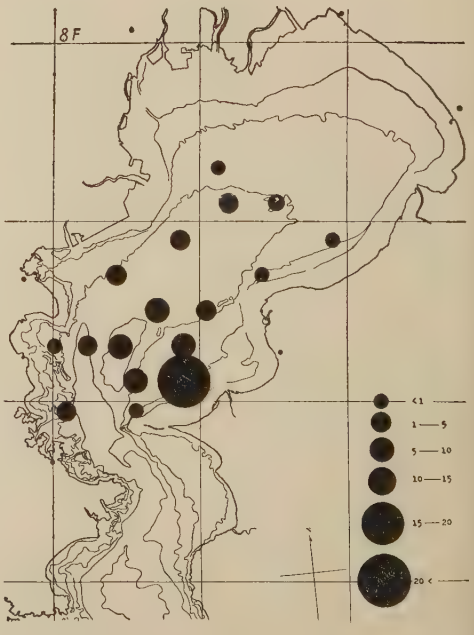
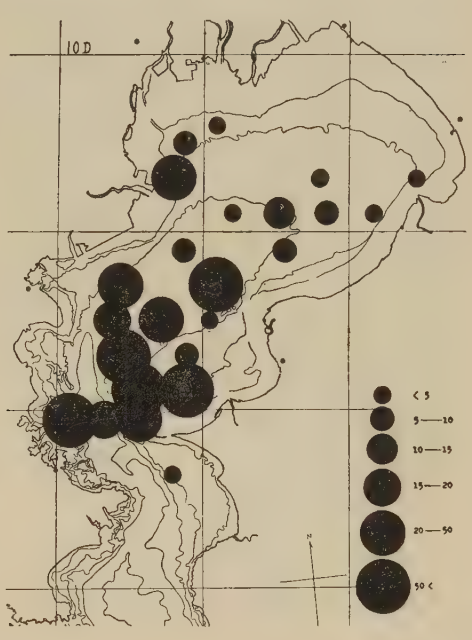
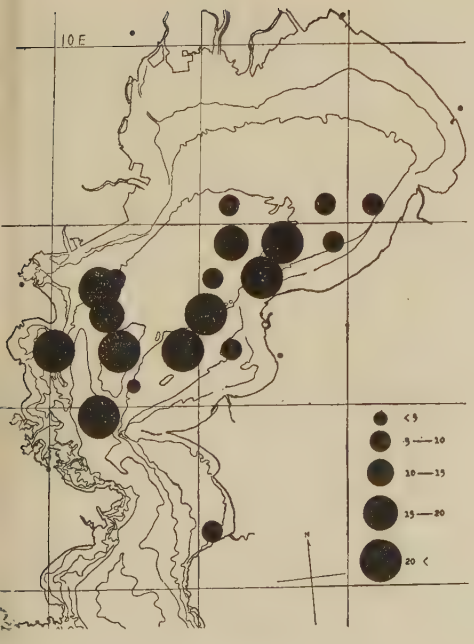

8F
< 1
1 — 5
5 — 10
10 — 15
15 — 20
20 <
N

Fig. 9. *Pilumnoplax vestita*. For A–F, see Fig. 7.

Fig. 10. *Charybdis bimaculata*. For A–F, see Fig. 7.

10C
2—5
6—10
11—15
16—20
20 <


10D
< 5
5—10
10—15
15—20
20—50
50 <

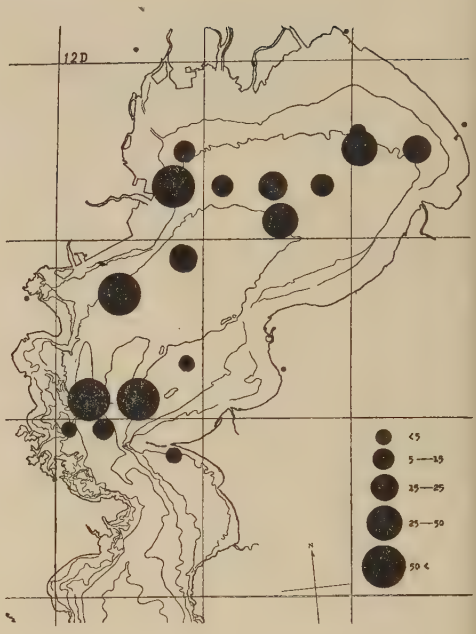

10E
< 5
5—10
10—15
15—20
20 <

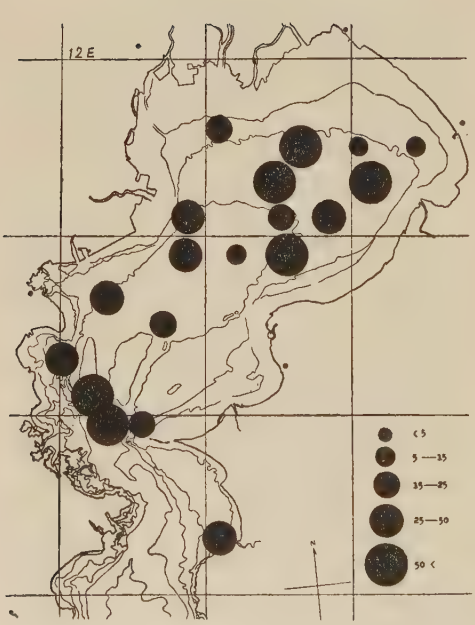
10F
< 5
5—20

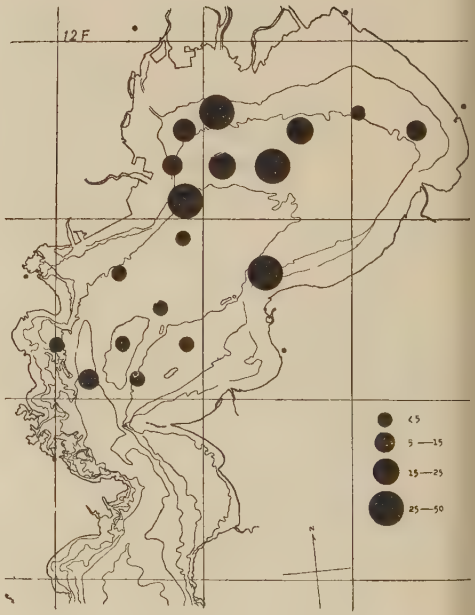
Fig. 11. *Trachypenaeus curvirostris*. For A–F, see Fig. 7.

Fig. 12. *Squilla oratoria*. For A–F, see Fig. 7.

12C
1—2
3—4
5—6
7—8


12D
<5
5—15
15—25
25—50
50<


12E
<5
5—15
15—25
25—50
50<


12F
<5
5—15
15—25
25—50

(e) *Charybdis bimaculata* (Miers)

This small portunid crab widly occurs on sandy or mudy bottoms of Tokyo Bay. The distributions of this carb basing on the material (Tables 12–17) taken at seasons of late spring, late summer, and late autumn of 1954 are schematized in the Fig. 10, A–F. From those figures, it may be seen that the species is mainly distributing on the area-B and about southern half of the area-A. Also it may be known that the animal in this bay is most abundant at late spring, least at late summer, and somewhat less at late autumn than at late spring at least among those seasons, on which surveys were made. The distribution in the area-B is more dense than in the area-A. This tendency is similar to that found in the cases with *Crago affinis*, *Crangon japonicus*, *Plumnoplax vestita*, and others.

(f) *Trachypenaeus curvirostris* (Stimpson)

This medium sized penaeid is an important species as an object of shrimp-fishery of this bay. As seen in the Tables 12–14 and Fig. 11, A–F, this shrimp occurs much in the area-B and in the middle part of the area-A, especially in the former. It seems that the shrimp appears most abundantly in late autumn. In this season, it is collected at 9 stations in the area-A, at 8 stations in the area-B, and at 2 ones in the area-C. In the late spring, it appears to be scarce in the area-A (Fig. 11A). Most specimens of this season was caught in the area-B. Whilst in the late summer, those were sequred much fewer in comparison to late spring. As seen Fig. 11, B and E the shrimp was collected from only 3 stations in the area-B, and 2 ones in the area-C.

(g) *Squilla oratoria* De Haan

A mantis shrimp, *Squilla oratoria* is an important species in Japan as being available for human food. It is distributed all over the bay as shown in the Fig. 12. A noteworthy seasonal change of its distribution is found to exist. In late spring and late summer, it appears comparatively much in north-east part of the bay (Fig. 12, A, B, D, and E). But in late autumn, it becomes scarce in the part said above, and it occurs abundantly on opposite side (Fig. 12, C and F).

(8) Discussion

With regard to the items: number of species, number of individuals, and weight per unit area of Tokyo Bay, the most high value is seen in the area-B, next high value in the area-C, and the lowest one in the area-A irrespective of season as mentioned already. Tokyo Bay accepts many rivers on its interior parts, namely on northwest coast of the area-A. Thus most numerous species as well as most numerous individuals must be found to exist in the area-A in ecologically speaking. Neverthless the fact is that as given above. It may be interpreted as that the waters of the area-A, especially shallow waters which afford good habitats for animals inhabiting such places, undergo considerable contamination by drainage of many cities including that of various factories.

In the present investigation, no crustacea have been collected from the St. 15, and it appears to be that the productivity of crustacea is small both numerically as well as gravimetrically in the vicinities of the St. 15. This tallies well with the results made by MASUI[5] and KAWAGUCHI[2]. According to MASUI, the place in question is very poor in benthic communities. KAWAGUCHI mentions that the place set forth has low animal density in spring time. This is probably due to that the place is in pollution by accumulation of contaminated substances drained from the cities as being the place is a stagnant area of sea water according to the result made by SUDA et al[11].

Number of species, and that of individuals per unit area are largest in late spring, smallest in late summer, and intermediate between those in late autumn. The fact that the number of individuals per unit area is largest in late spring is in correspondence with the nemtions made by KAWAGUCHI. With regard to weight per unit area, the sequence of the latter two seasons is inverse: it is largest in late spring, smallest in late autumn, and intermediate between those in late summer. The reason for this fact may be as that set forth already in p. 263 of this thesis.

Though there is no difference between both east and west halves of the bay in number of species inhabiting those areas, a remarkable disparity is found to exist between them in individual number per unit area. The number was found always larger in the east half throughout all samplings. The same trend was also found in the weight per unit area. In the result of MASUI's investigation, individual number per unit area is not discrepant between those east and west regions, but weight per unit area was found to be different in those two halves. Namely it was larger in the east half than in the west half. SHIMADZU and YAMANE[10] (p. 55) also state that the bottom fauna is richer in the east half dealing with material taken during May to October. These are the same as is the case with the present investigation.

In Tokyo bay, it is known that open-sea waters enter along its east side, and its coastal waters go out along its west side. Then it may be said that the west side is more polluted than the other side because of the drainage of many cities and various factories get out together with the costal waters along the west side of the bay. Accordingly it may be said that the matter aforementioned is showing a parallelism to the degree of pollution of the sea-waters.

Finally it should be added herewith that body-weight per individual is larger in the west half in comparison to that of the east one, although the distribution density is larger in the east half than in the other half as written above (P. 261, Table 7) throughout all samplings. This means that the individuals, which inhabit the west half, are larger in size than those living on the east half. It is, however, difficult to reveal the reason of the fact within the scope of the present investigation.

Summary

(1) In total 84 species were collected, 10, 21, and 46 species out of them are referred to anomura, macrura, and brachyura respectively. The rest belongs

to stomatopoda, amphipoda, and isopoda.

(2) Average number of species, that of individuals, and average weight per unit area of each area are: area-B>area-C>area-A.

(3) Both average number of species and that of individuals per unit area of each season are: spring>autumn>summer.

(4) Mean weight per unit area of each season is: spring>summer>autumn. But that of individual per unit area of each season is: summer>spring>autumn.

(5) Both average number of individual per unit area, and average weight per unit area are larger on the east half of the bay.

(6) The following 9 species are found to be dominant: *Crago affinis* (De Haan), *Heptacarpus minutus* (Yokoya), *H. rectirostris* (Stimpson), *Crangon japonicus* (Miers), *Pilumnoplax vestita* (De Haan), *Chrybdis bimaculata* Miers, *Trachypenaeus curvirostris* (Stimpson), *Latreutes planirostris* (De Haan), and *Squilla oratoria* De Haan. (Written in order of abundance).

(7) In crustacean fauna, the area-B is nearer to the area-C than to the area-A.

(8) No crustacea are obtained throughout all samplings, made at late spring, late summer, and late fall, in the St. 15, south-east off Tokyo and east off Ōmori.

(9) Some seasonal changes are found to exist in distribution of such important species as *Crago affinis*, *Crangon japonicus*, *Pilumnoplax vestita*, *Charybdis bimaculata*, *Trachypenaeus curvirostris*, and *Squilla oratoria*.

References

1) Hamai, I. 1955. Stratification of community by means of "Community coefficient". Jap. J. Ecol., 4 (4) 171–175 (in Japanese with English synopsis).

2) Kawaguchi, M. 1946. A record on the benthic animals of the Tokyo bay. Contr. Physiol. Ecol., (85) 1–9, 1 Pl. (in Japanese).

3) Kubo, I. 1951. Some macrurous decapod crustacea found in Japanese waters, with descriptions of four new species. J. Tokyo Univ. Fish., 38 (2) 259–289.

4) ———. 1955. On Leptochelan shrimp in Japan. Bull. Biogeog. Soc. Jap., vols. 16–19, 98–106 (in Japanese).

5) Masui, T. 1943. On benthic communities of the Tokyo Bay. J. Oceanog. Soc. Jap., 3 (2) 130–141 (in Japanese).

6) Motomura, I. 1932. On a statistical treatment of animal association. Dobutsugaku Zasshi (Zool. Soc. Jap. Tokyo), 44 (528) 379–383 (in Japanese).

7) ———. 1947. Further notes on the law of geometrical progression of the population density in animal association. Physiol. and Ecol., 1 (2) 55–60 (in Japanese with English synopsis).

8) Ōtuka, Y. 1936. The faunal character of the Japanese pleistocene marine mollusca, as evidence of the climate having become colder during the pleistocene in Japan. Bull. Biogeog. Soc. Jap., 6 (16) 165–170.

9) Sakai, T. 1936–39. Studies on the crabs of Japan (I–II), pp. 1–741+1–23, 111 Pls. Yōkendō (Tokyo).

10) Shimadzu, T., and K. Yamane. 1948. Studies on the benthos of Tokyo Bay (1). On the seasonal change of temperature of bottom mud with reference to the benthos. Bull. Jap. Soc. Sci. Fish., 14 (1) 51–55 (in Japanese with English synopsis).

11) Suda et al. 1931. The results of the oceanographical observations on board R.M S. "Syunpū Maru in the Tokyo-wan in the spring, 1929. Kaiyo-ji-hō (J. Oceanog. Imp. Mar. Observ. Kōbe, Japan, 3 (1) i–iv+1–119. (in Japanese with English abstract).